MORBID GROWTHS & SPOROZOA.

J. Jackson Clarke.

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CANCER, SARCOMA,

AND

OTHER MORBID GROWTHS

CONSIDERED IN RELATION TO

THE SPOROZOA.

BY
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PREFACE.

THE observations and ideas recorded in the following pages represent the work of about three years, and include my papers read before the Pathological Society in the Session 1892-3, with the exception of that describing the gregarina-adenoma of the cat's lip which forms part of a sketch of the biological basis of the conclusions I have arrived at. To this sketch, printed in the *Medical Press and Circular*, Aug. 16, 23, and 30, 1893, I would refer readers who may not have time to study the matter more fully in the works of Dr. L. Pfeiffer. The publication of my work at the present moment is of the more importance since in every essential point I am confirmed in my beliefs by more recent observations.

For the material on which I have worked I am indebted chiefly to members of the surgical staff of St. Mary's Hospital, and to Mr. Crowle, the surgical registrar.

In the expression of my views I have received most signal and unstintedly generous help from my friend and colleague Mr. E. W. Roughton, whose skill in photography has been of immense service to me, without in the least committing Mr. Roughton to my views.

My observation of sporozoa in cancer was made independently, and was the direct outcome of the study of a case of psorospermosis of the urinary tract. It is interesting to observe that the dense deeply-staining cells, and the spore-cysts

I recognised in cancer as being identical with the psorosperms of the ureter, are regarded by pathologists in this country as degenerated material.

To pathologists who, like myself, had long felt affronted by the gross and still inscrutable lesions of cancer, and to curators of museums who had found their sense of order, but not their intellect, satisfied with arranging on shelves typical specimens of cancer, adenoma, and sarcoma, the light which has recently been shed on the nature of new growths must have been highly welcome. The rays of this light are as varied of hue as those of the spectrum. The earliest, that of L. Pfeiffer, has not failed, but grown stronger. The work of Podwyssoski and Sawtschenko would appear to have been inspired by that of L. Pfeiffer, while that of Nils Sjöbring, which came after Wickham's, seems to have been independent. The light of the Collège de France, which arose independently with Malassez, and was transmitted to Darier and Wickham, awakened another fire at the Institut Pasteur, whence a strong and brilliant beam shot forth from the brain of Soudakewitch and received the weighty support of Metchnikoff. The fire of Soudakewitch has lighted many others, and among them one in England, where the brilliancy of the beam has not only been sustained, but, in the able hands of M. A. Ruffer and his colleagues, has been amplified and strengthened, and so much so that many would appear to regard the parent fire of the Collège de France as quite eclipsed.

I feel it my duty to record my view of these matters, to add a little light of my own, and to help to draw together the variously coloured rays into white light.

J. JACKSON CLARKE.

ST. MARY'S HOSPITAL MEDICAL SCHOOL,
October, 1893.

CANCER, SARCOMA, AND OTHER MORBID GROWTHS,

CONSIDERED IN

RELATION TO THE SPOROZOA.

RECENT WORK RELATING TO CANCER AND OTHER AFFECTIONS.

Within the past few years, the results of many important inquiries into the nature of cancer and other new growths have been published.

The most important part of the work to be considered is of a pathological character, either experimental or histological. The aim of most investigations has been to prove or disprove a parasitic causation for cancer and sarcoma. Many valuable clinical observations have also been published which bear on the question but only indirectly, so that I shall omit them from this brief survey.

As long ago as 1847 Virchow[1] and others[2] described cells lying surrounded by firm capsules within other cells of cancerous growths.

In 1851[3] Virchow summed up his observations on these intracellular cells thus :—

" A portion of a large cell with granular contents, perhaps an altered nucleus, the dimensions of which it has, becomes homogeneous and clear, like water. This portion has at first a sharp and stout wall, which very soon by the addition of fresh layers is thickened and becomes doubly contoured in every way like a cartilage cell."

In another part of the same paper Virchow figures in the cell-nests of a squamous epithelioma clusters of minute rounded bodies which had also been noticed by other observers. Virchow regarded the intracellular cells as the endogenous offspring of the cells containing them.

Virchow and his followers regard all the constituent cells of cancer and sarcoma as lineal descendants of cells which once formed part of the body of the animal in which the tumour grew.

The accuracy of Virchow's description of the histological structure of cancer and sarcoma has been proved again and again. The only question with regard to them is a question of interpretation.

Cohnheim's well-known hypothesis as applied to cancer is thus expressed[4] by the distinguished patho-

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[1] Virchow. "Virch. Arch.," vol. i.

[2] Lebert. " Phys. Path.," pl. xviii, fig. 8, &c. ; John Hughes Bennett on " Cancerous Growths," p. 32.

[3] Virchow. " Virch. Arch.," vol. iii.

[4] Cohnheim, loc. cit. p., 817.

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logist who so lately as 1884 passed away:—"In the tumours the redundant germinal material is inherited just as a sixth finger or a thirteenth rib: and possibly, too, the feeble resisting power of the tissues is transmitted to the offspring." At the present time this theory is challenged on all sides.

The interesting observations and theories of Klebs[5] in Germany and Creighton[6] in England merit careful consideration, and will be referred to in the section on cancer of the breast and elsewhere.

Bacteriology has proved that for some of the new growths it is not true that their constituent cells are all lineal descendants of the tissue-cells of the individual in whom the growth has arisen. Such growths Virchow has included in a group, the "infective granulomata." A glance at a section of a leprosy nodule of the skin suffices to show in what numbers these parasitic unicellular fungi may be present.

Pathologists have tested cancer by the bacteriological methods; in the majority of cases the results have been negative, or have been shown to be erroneous where microphytes were accounted to be the cause of the disease.

Many authors have described schizomycetes in cancer. Scheuerlin,[7] Koubasoff,[8] and Russell,[9] have definitely asserted that they were the cause of the disease.

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[5] Klebs. Allgemeine Pathol., Bd. ii, p. 524.

[6] Creighton. "Infection of Connective Tissue in Cancer of the Breast." 1879.

[7] Scheuerlin. "Berlin. Med. Soc." 1887.

[8] Koubasoff. "Med. Cong. St. Petersburg." 1889.

[9] Russell. *Brit. Med. Journ.* 1890.

The labours of Shattock and Ballance, of Senger,[10] and Klein,[11] have shown these conclusions to be erroneous.

The work of Russell deserves more detailed notice. By staining sections of cancer with carbol-fuchsin, and after staining with iodine green, certain minute bodies ("fuchsin bodies") were picked out a bright red colour, and from their form Russell concluded that they were fission-fungi. One figure in the author's paper represents a large cancer cell containing another, in the interior of which are four "fuchsin bodies." The included cell in this figure is what many now regard as an intra-cellular psorosperm, and the "fuchsin bodies" as spores. The accuracy of Russell's figures is now fully established; and though the "fuchsin bodies" have proved not to be fungi, Russell's earnest work has done much to stimulate fresh inquiry into the nature of cancer.

L. Pfeiffer[12] was the first to publish a description of parasitic protozoa in cancer.

On teasing out a fragment of melanotic cancer on the warm stage, Pfeiffer saw plasmodia in active amœboid movement, and cells resembling sporozoa.

In a recent paper this author comparing the structure of tissues infected respectively by cancer, sarcosporidia, myxosporidia, and microsporidia, points out that the four cases are parallel; in each a double process of growth is present, partly heterologous, *i.e.*, parasitic, partly homologous, *i.e.*, belonging to the tissues of the host.

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[10] Senger. "Berlin Med. Soc.," Feb., 1888.

[11] Klein. Beitäge zur Pathol. Anat., Bd. xi, p. 127.

[12] L. Pfeiffer. "Correspondenzblaetter des Allg. aertzlich Vereins," Thuringen, 1888 and 1893.

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L. Pfeiffer has given the world two invaluable comprehensive works bearing on the subject, these are the "Protozoen als Krankheitserreger," 1891, and "Untersuchungen über den Krebs," 1893. Both works are full of original observations, and at the same time record faithfully the labours of others in the same field.

The peculiar bodies found in molluscum contagiosum had already by many authors, including Virchow, 1865, been compared to gregarinida. Bollinger[13] first strongly maintained that they were parasites, and compared them to bodies met with in a similar affection in birds. Neisser[14] and Pfeiffer[15] supported in detailed memoirs Bollinger's views, and attributed the disease to the parasites. Kaposi, Duhring, Török, and Tommasoli[16] regarded the bodies as products of degeneration, and could not discover in them the characters of coccidia.

A. Q. Silcock[17] published a case of acute psorospermosis associated with intestinal papillomata. A woman, æt. 53, was admitted into St. Mary's Hospital under Dr., now Sir Wm., Broadbent in July, 1889, with fever and a swollen tender liver. Death soon followed. At the autopsy inflammatory areas and five papillomata with inflamed bases were found in the small intestine, and the liver and spleen were studded with pale spots which Silcock found to contain psorospermiæ somewhat resembling the rabbit's coccidia save that they were much smaller. Cultivation gave a formation of spores. The histology showed nodules surrounded by an inflammatory zone, and containing numerous parasites and amœbid spores.

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[13] Bollinger. "Virchow's Arch."

[14] Neisser. "Vierteljahrschrift für Dermatol." 1883.

[15] Pfeiffer. "Zietschrift für Hygiene." 1888.

[16] Török. "Monatshefte für Dermatol." 1890, No. 4.

[17] A. Q. Silcock. "Path. Soc. Trans." 1889.

In 1889 R. Thoma,[18] of Dorpat, described within the nuclei of epithelial cells in cancers abundance of bodies resembling sporozoa.

Malassez, who for ten years had been working at the subject, described in 1889, in a cancer of the jaw shown by Albarran[19] before a Paris society, bodies which he recognised as coccidia. Darier[20] in the same year described similar bodies in a rare disease previously known as "Ichthyosis sebacea," and now rightly named "Darier's disease," in recognition of the author's valuable work. Darier also recognised similar bodies in Paget's disease. Darier's disease gains in interest from the fact that in some cases it terminates in epithelioma. His material came from two cases in the wards of Prof. Fournier. Both patients, a man and a woman, were past middle age. The lesions were confined to the skin and were very widespread. At first they consisted of papules formed by the accumulation of a substance resembling sebum in the mouth of the follicles ; this was followed by some inflammation, and in one patient by epithelioma in the pubic region. The course of the disease was extremely slow.

Microscopically some of the cells of the rete were found to contain large clear cells with definite capsules. In the superficial layers these intra-cellular cells were replaced by dense, highly refracting grains.

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[18] R. Thoma. "Fortschritte der Medicin," 1889, No. 11, June, p. 413.

[19] Albarran. "C. R. Soc. de Biol." 1889.

[20] Darier Ibid.

Darier regards the disease as a psorospermosis, and the intra-cellular cells and bright grains as psorosperms. Darier also first described psorosperms in Paget's disease, and he was able to observe the process of sporing in cultures.

In the same year followed L. Wickham, whose observations have been embodied in "A Contribution to the Study of Psorospermosis of the Skin, and of certain forms of Cancer."[21] Wickham's description of Paget's disease is complete in every way, and includes a full account of all the work that has been published on the subject since the individuality of the affection was first recognised by Paget in 1874. The author gives beautiful illustrations of the histology of his cases, and of another, Crocker's, in which a similar condition was present in the scrotum. He describes large encapsuled nucleated cells placed within epithelial cells, and encapsuled collections of spore-like bodies. The author regards these as psorosperms, and from their distribution in the tissues he considers them as the cause of the disease. Wickham looks on cancer as the third stage of the disease. The cancer may be of the squamous or of the tubular variety, and it contains psorosperms in great abundance. Wickham concludes that the cancer of Paget's disease is due to psorosperms.

Darier and Wickham's observations on Paget's disease have the countenance of Balbiani and Malassez, and have been confirmed in England by Bowlby[22] and J. Hutchinson, jun.[23] Thin, in connection with Bowlby's paper, referred the cell-inclusions of Paget's disease to degeneration, an opinion with which Ruffer[24] and Walker have since expressed agreement. I may state here that the forms figured by Wickham are to be found in most squamous epitheliomata, and the fidelity and freedom from systematisation of the illustrations accompanying his paper I have been able to verify amply. Many of the bodies figured by Wickham I have been able to find in the psorospermial cysts of the ureter.

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[21] Wickham. "Psorospermoses Cutanées," Paris. 1890.

[22] A. A. Bowlby. "Trans. Medico-Chirurg. Soc." 1890.

[23] Jon. Hutchinson, jun. "Trans. Path. Soc." 1890.

Nils Sjöbring[25] was studying karyokinesis in sections of mammary cancers, hardened in alcohol and stained in Ehrlich's acid hæmatoxylin and carbolic eosin, when he observed many bodies identical in form with microsporidia, as figured by Balbiani and Bütschli. The bodies were stained more deeply by the eosin than the protoplasm of the cells which contained them. Some appeared to be within the nuclei, where they were, as a rule, surrounded by a space. Sometimes several were found within one nucleus. The larger parasites lay within the cell protoplasm, the nucleus being pushed aside. The parasites varied in size from 2—15 μ. They frequently contained chromatin filaments. Sjöbring regarded the bodies described by him as differing from those figured by Wickham.

Von Heukolom[26]. examined 200 cancers, and his observations agree in the main with Sjöbring's. The latter insists that the bodies in question exhibit

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24. Ruffer and Walker. "Journal of Pathol," vol. i, No. 2, p. 201, Oct. 1892.

25. Nils Sjöbring. "Fortschritte der Medicin." 1890, July 15th.

26. Von Heukolom. "Centralblatt für allgemeine Pathol." 1890 No. 22.

evidences of a life-history which absolutely excludes cell degeneration as an explanation of their presence.

Von Kossinsky[27] has also published a valuable study of cell-inclusions in cancer. Without being able to express a definite opinion as to the nature of the bodies referred to, he agrees with Klebs[28] that the phenomenon regarded by many as spore-formation differs from accumulation and disintegration of leucocytes met with in ordinary inflammations.

Vincent[29] demonstrated in cancers bodies resembling coccidia, and described methods of staining.

Billroth[30] says that there is little doubt that molluscum contagiosum and the similar disease of birds are due to sporozoa. In comparing tuberculosis and cancer he says it is clear that the sporozoa have the property of determining formative processes in animal cells.

The investigations of Shattock and Ballance[31] have been of considerable importance; like Klein[32] and Senger, they show that Russell's position is untenable. They found careful cultivation experiments gave entirely negative results. Incubation of fragments of cancer showed in sections wandering of nuclear matter. Also inoculation experiments with monkeys, dogs, and other animals, conducted with the utmost

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27. Von Kossinsky. "Ueber Physaliphoren in Krebsgeschwülsten." Warsaw, 1890.

28. Klebs. Loc. cit. supra.

29. Vincent. "C. R. Soc. de Biologie," March, 1890.

30. Billroth. "Ueber die Einwirkung lebenden Pflanzen-und Thierzeilen" Wien. 1890.

31 Shattock and Ballance. *Brit. Med. Journ.*, 1891; "Proc. Roy. Soc.;" "Trans. Path. Soc." 1888. &c.

32. E. Klein. "Beiträge zur Path. Anat.," Bd. xi, p. 125.

care and skill, gave always negative results. With admirable impartiality they review the work done on cancer up to the year 1891. And they relate Hanau's, Nerinsky's, Eiselberg's, and Hahn's successful inoculation experiments. They conclude that though cancer is in all probability an infective disease, the nature of the infection is still unknown. In one of their communications ("Path. Trans.," 1888) the authors delineate the histological appearance of a piece of human scirrhus after eight days' incubation. The bodies there drawn and termed nuclei have a great resemblance there to amœboid psorosperms, and I think it probable that thus is shown a spore-formation on the part of parasites similar to that obtained by Darier in the "grains" he incubated from one of his patients.

Walter K. Sibley[33] mentions cases of human sarcoma successfully inoculated on a dog by C. O. Weber.

Steinhaus[34] described the sporozoa-like bodies of cancer. This paper is illustrated. The intra-nuclear and other cell-inclusions which the author is prepared to admit as sporozoa closely resemble some of those figured by Nils Sjöbring.

A. Borrel[35] criticised chiefly the work of Darier and Wickham. He finds in sections of squamous epithelioma large encapsuled intra-cellular nucleated cells, which are sometimes replaced by a mass of nuclei apt to be "mistaken" for spores, and also "reticulated globes figured but not described by Wickham." According to this author, these globes are due to a peculiar mode of degeneration and the intra-cellular cells are insufficient to justify the diagnosis of coccidia.

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[33] W. K. Sibley. "Trans. Path. Soc.," 1871: "Non-contagiousness of Cancer."

[34] Steinhaus. "Virch. Arch.," 1891, p. 533.

[35] A. Borrel. "Archives de Médecine expt.," 1890, vol. ii, p. 788: "Sur la signification des figures décrites commes coccidies dans les épithéliomes."

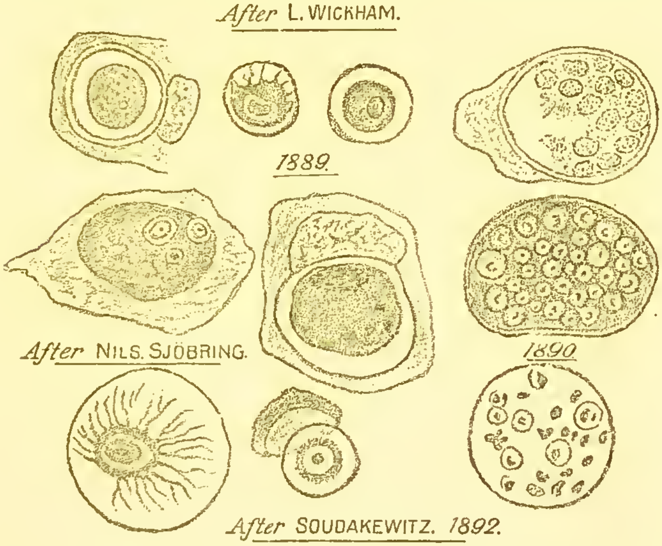

FIG. 1.—In three horizontal rows are some of the bodies depicted in cancer as parasites by the authors named. The second figure from Wickham represents, I believe, a body of the same nature as the first of those taken from Soudakewitch. The fourth of Wickham's I shall endeavour to show to be a form of swarm-sporing, it is what Borrell has termed "reticular degeneration." The third figure from Sjöbring is identical with Virchow's "nests" of daughter cells of cancer, the first from the same author shows similar cells within the nucleus of an epithelial cancer, and goes far to prove these bodies to be sporozoa. The three drawings to the right represent, I believe, different phases of sporing.

Sheridan Délépine,[36] whose work on the psorosperms of the rabbit's liver has done so much to awaken an interest in, and to help in the formation of a sound basis for, work on the pathogenic *rôle* of sporozoa, concludes[37]: " The evidences I have been able to collect are against the psorospermial nature of the bodies which I have observed in epithelial tumours, and I think that the same conclusions must also extend to the bodies observed in Paget's disease of the breast." In the last quoted article Délépine describes within the epithelial cells of the ducts of a human cancerous liver bodies resembling coccidia. He depicts a section of a duct of a cancerous liver with " one of the cells, and perhaps two others, contain besides the nucleus a body which seems encapsulated, and which in the fresh state closely resembled a young psorosperm."

E. Klebs[38] opposes the view that cancerous tissue contains sporozoa, and considers the bodies described as such to be blood-corpuscles, products of degeneration, &c.

Firket[39] and Schütz[40] have taken up a position similar to that of Klebs.

Cornil[41] considers that karyokinetic processes have been mistaken for spore formation.

Hansemann[42] has found irregular mitoses in epithelia other than those of cancer, and from the fact that the

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36. Délépine. " Trans. Path. Soc.," vol. xli, 1890; vol. xlii, 1891.

37. Délepin. " Trans. Seventh Internat. Congress of Hygiene,' London, 1891, p. 93.

38. Klebs, " Deutsch. med. Woch." 1890, No. 32.

39. Firket. " Centralblatt für allg. Pathol." 1890, No. 20.

40. Schütz. " Münch. med. Woch." 1890, No. 35.

41. Cornil. " Journal de l'Anat. et de Phys." 1891, No. 1.

42. Hanseman. " Virch. Archiv." 1891.

irregular processes of cell-division and "so-called" spore-formation chiefly met with in the degenerated parts of cancers, suggests that they are due to want of nutriment. This observer agrees in the main with Cornil.

Fabre-Domergue[43] identifies the bodies described as sporozoa, some with those described by Virchow as cells of endogenous origin, others with bodies described by the same author as degeneration products.

Duplay and Cazin,[44] summing up their investigations on the work of Nils Sjöbring and others, regard the parasitic nature of the bodies as not proven.

G. Hauser[45] discusses the bacillus theory. The author regards the identity of the structure of the secondary growths wherever occurring to contradict the bacillary hypothesis.

Kiener[46], Professor of Pathology at Montpellier, has expressed himself as sceptical, and influenced Borrel's first article.

Ribbert[47] could not detect the crooked bodies described as spores by Nils Sjöbring. Ribbert comes to the conclusion that the bodies are due to a metamorphosis of the nucleus, the granular bodies being the remains of nuclei or portions of chromatin.

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[43] Fabre-Domergue. "Les Coccides dans les Néoplasmes," "Congrès de Chirurgie," Paris, 1891.

[44] Duplay and Cazin. "Trans. Seventh Internat. Congress of Hygiene," vol. ii, p. 81, London, 1891.

[45] G. Hauser. "Das Cylinder Carcinom des Magen und Dichdarms," p. 124. 1890.

[46] Kiener, referred to by Borrel. "Arch. de Médecine expérimentale," vol. ii, 1890, p. 893.

[47] Ribbert. "Deutsch. med. Woch."

Virchow[48] had not been able to observe anything in cancer which pointed to a parasitic origin.

Another supporter of the view of degeneration may be taken, Welch of the Johns Hopkins Hospital, quoted by Nœggerrath as coming to the following conclusions :—"That the parasitic nature of the cell-inclusions is not proved, but, on the contrary, they are of the following characters : (1) Portions of cells keratinosed centrally. (2) Masses of kerato-hyalin. (3) Included leucocytes with degeneration or fragmentation of nucleus. (4) Fragments of nuclei of leucocytes."

Strœbe[49] admits the close resemblance of some of the cells of cancer to sporozoa, but leaves open the question of identity.

Ramsay Wright,[50] like Russell, thinks the bodies described as sporozoa belong to the saccharomycetes.

E. Nœggerrath[51] has ably reviewed the whole subject in a work to which I am greatly indebted. He confirms all the descriptions given by Nils Sjöbring, and up to a certain point was prepared to entertain the conclusions of the latter ; but in a section of cancer, stained first with carmine and then by Gram's stain, he found an appearance as if part of the nucleus was undergoing liquefaction, and concluded that most of the intra-cellular bodies arose in this way, and were not parasites. Some of the cell-inclusions, however, resembled protozoa closely, and the author is prepared to admit them as such. But these forms are so few that they "cannot have any important *rôle* in cancer." He observed the subdivision of large cells in cancer, and the migration of the resulting particles—the swarm-spore formation of many authors. Reference is made to Ehrlich's observations on the erythrophile and cyanophile portions of the nuclei of cells. The work is one of the most valuable published on the subject. The illustrations are excellent. Many will feel that, had the author been more practised in the biology of the sporozoa, he would have come to a different conclusion.

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[48] Virchow, in a note on Steinhaus's article. "Virchow's Archiv." No. 127, p. 188. 1892.

[49] Strœbe. "H. Ziegler's Beiträge." 1891.

[50] Ramsay Wright. "Centralblatt für allgemeine Pathol." 1890, No. 11.

[51] Nœggerrath, "Beiträge zur Struktur und Entwickelung des Carcinoms," Wiesbaden, 1892.

Podwyssoski and Sawtschenko[52] give complete references to previous work, and describe, with drawings, parasites which they found most abundantly in various cancers. The forms are the encapsuled intracellular cell, the conglomerated masses of minute cells (spores), and small cells within the protoplasm of epithelial cells. They thought that these forms warranted the positive conclusion that they were parasitic sporozoa, but they could not from the histological characters alone decide as to whether they were ætiological factors of the disease.

Later, Sawtschenko[53] traces the life-cycle of the parasites in a case of cancer of the lip. He finds evidence that the capsules are secreted by the parasite and not by the host-cell, and is inclined to regard the parasites as the cause of disease. Some of the illustrations by Podwyssoski show peripheral sporing.

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[52] Podwyssoski and Sawtschenko. "Centralblatt fur Bacteriologie," &c., 1892, xi, Nos. 16—18.

[53] Sawtschenko. Ibid., July 5th, 1892; "Weitere untersuchungen uber schmarotzende Sporozoa in den Krebs-geschwulsten," 1891.

Soudakewitch[54] gives detailed descriptions of cell-inclusions he met with in ninety-five cancerous growths, with three plates. These cell-inclusions, the author found, could not possibly be the result of degeneration. He found them most abundantly in cancer of the liver. The appearances described led the author to say that in every case parasites of the nature of sporozoa were present.

Metchnikoff,[55] in a note on Soudakewitch's manuscript, expresses the decided opinion that the cells met with and described by Soudakewitch are parasites akin to the rabbit's coccidia. The same author published (*Brit. Med. Journ.*, Dec. 10th, 1892) an independent article which has done much to arouse interest in this matter.

In a later article Soudakewitch[56] continues his work and gives additional figures, which, like those of the first article, are of great beauty, and after having verified almost every one of his descriptions, I can also say of complete accuracy. Soudakewitch regards the parasites as sporozoa, and thus describes the liberation of swarm-spores :—"The destruction of the cancerous cells is followed by the liberation of the parasite of which the capsule ruptures, and the spores, escaping, infest the neighbouring cells."

G. Sims Woodhead, in the "Morton Lecture on Cancer" of 1892 (*Lancet*, May 7th, 1892), describes en-

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[54] Soudakewitch. "Annales de l'Institut Pasteur," tome vi, No. 3, p. 145.

[55] Metchnikoff. Ibid., p. 158.

[56] Soudakewitch. Ibid., No. 8, Aug. 25th, 1892.

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capsuled cells, some with a stellate arrangement of protoplasm situated within epithelial cells of cancers, and closely resembling coccidia. The author also alluded to Soudakewitch's observations, and mentioned that in some sections submitted to him by Ruffer and Walker, he found bodies with all the characteristics of coccidia, and absolutely distinguishable from blood-corpuscles and products of degeneration. "In some of the capsules there appears to be a process of division going on, whilst here and there are capsules in which several (four to twelve) small rounded or crescent-shaped psorospermiæ,[58] in other respects like the coccidia except that they have no capsule, may be made

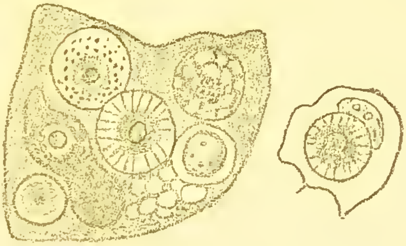

FIG. 2.—To the left a cell of a cancer of the pancreas, containing several parasites of different forms; one of them is intranuclear, after Soudakewitz;[57] to the right a cell of a squamous epithelioma after Borrel. It contains a body which the author regards as not parasitic. It is identical in structure with one of those depicted as a parasite by Soudakewitch.

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57. Soudakewitch. "Centralbt. f. Bakteriol," vol. xiii, Nos. 14-15. 1893.

58. Sims Woodhead has since (1893) stated that this observation was erroneous.

out. In one or two instances, these appear to be lying free, between the epithelial cells, from which they have probably escaped on the breaking down of the mother capsule. They are of various sizes, from the diameter of a red blood corpuscle to three or four times that size. . . . We are still very far from the proof that these organisms are the actual cause of cancer."

In September, 1892, at the International Dermatological Congress held at Vienna, a discussion of the nature of Darier's disease and molluscum contagiosum took place.

Boeck (Christiania) thought the peculiar bodies met with in Darier's disease were the result of hyperkeratosis.

Neisser (Breslau) thought Wickham's conclusions with regard to Paget's disease untenable ; he thought Darier's disease had much in common with molluscum contagiosum, and the latter he had no doubt was due to coccidia. The author showed preparations in which spores were visible.

Ehrmann (Vienna) had found in pemphigus foliaceous cells similar to those described as coccidia in cancers. He thought it utterly impossible that two so different diseases could be evoked by the same cause, and, therefore, he regarded the psorosperm-like bodies in these diseases as degeneration products from epidermal cells. He shared Neisser's views with regard to molluscum contagiosum.

Ianovsky (Prague) related a case of Darier's disease in which healing took place in some parts whilst extension was going on in others.

Schwimmer recommended energetic cauterisation in the treatment of the disease.

Török explained the position taken up by Tommasoli and himself. On account of the failure of cultures, and chemical and anatomical differences between them and rabbits' psorosperms, they supported the cell-degeneration theory. The bodies resembling psorospermia in Paget's disease were nothing more or less than epithelial cells enormously increased as the result of inflammatory proliferation. A number of mitoses were "giant mitoses." Török regarded the psorosperm-like bodies in cancer as epithelial cells and white blood-corpuscles.

Jackson Clarke[59] published a case of psorospermosis of the urinary tract, giving references to similar cases by Lindemann, Eve, Bland Sutton, and Targett. The article is illustrated, and reasons are given for regarding certain bodies as parasites belonging to the sporozoa.

Since this case was the starting point of my work on this subject, it may be well to state a few of its more important features, especially as the other cases of the kind on record are not described fully. The contents of the uretal cysts when examined in the recent state were found to include bodies composed of numerous globules exactly resembling oil but proving to be of an albuminous nature when treated with alcohol and ether. When hardened in Müller's fluid and examined in sections, the contents of some of the cysts seemed to be an uniform mass looking like colloid matter. In others, oval bodies were present, embedded in a similar material. These oval bodies which at first I took to be degenerated cells because they resembled exactly cell-forms familiar to me in certain cancers, on closer

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[59] Jackson Clarke, "Trans. Path. Soc.," 1892, p. 94.

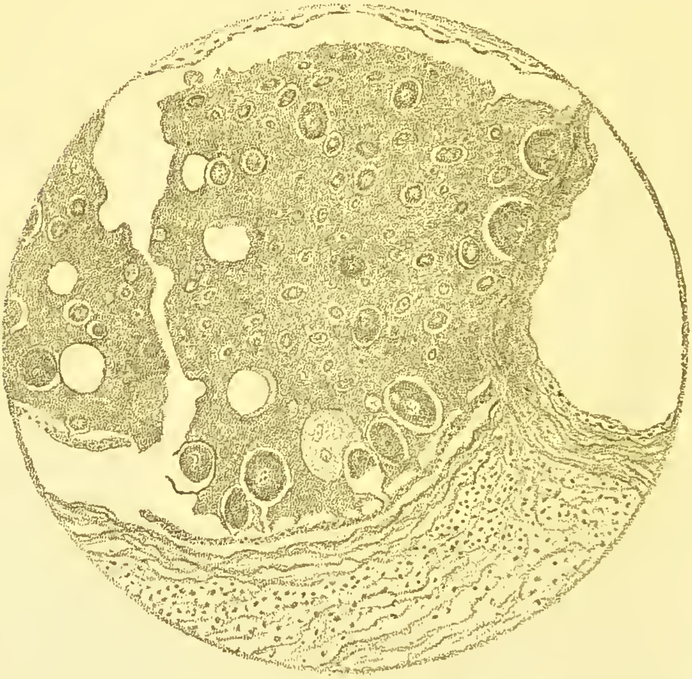

Fig. 3.—A section of one of the cysts of the ureter showing psorosperms embedded in albuminous material. Zeiss. D. oc. 2.

inspection proved to be sporozoa. The larger ones had a cell-protoplasm which stained deeply with carmine and hæmatoxylin, but yielded a colour differing from that of the tissue-cells. Their protoplasm had a dense texture and was highly refracting. They were larger than the coccidia of the rabbit's liver and a few only had capsules and these were very delicate. Most of the parasites were naked. Many of them were drawn out into fantastic forms and fused together. These, when stained, resembled colloid matter or other products of degeneration. Their nuclei were of the most

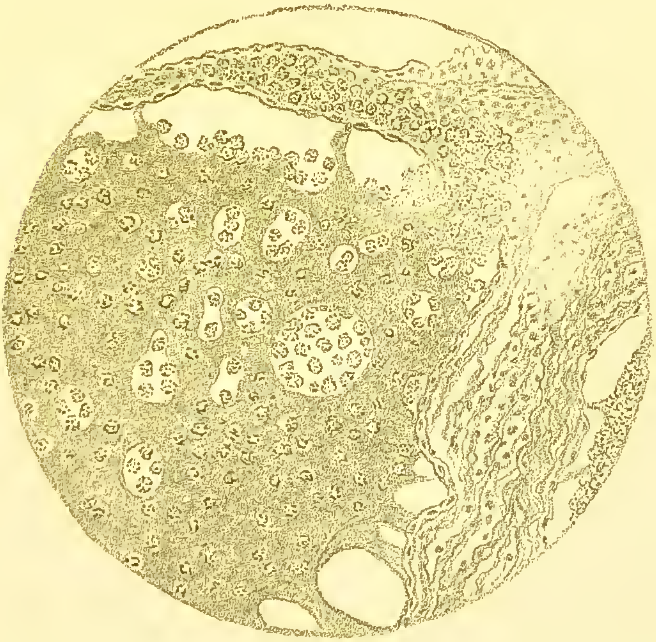

Fig. 4.—Section of another cyst in the same case. The oval psorosperms and also the albuminous material are breaking up into granular amœboid spores, many of which have passed into the connective tissues. Zeiss F. oc. 2.

varied aspect; some were vesicular with numerous nucleoli of characters common in sporozoa, others were indistinguishable in their reaction to hæmatoxylin from the nuclei of epithelial cells. Some of these were giant-nuclei. In some cases the parasites had several nuclei all staining well with hæmatoxylin.

In some of the larger parasites the nuclear and adjoining regions were completely occupied by roundish, granular corpuscles of very high refractive index. Many of these bodies were free in the cysts, Fig. 4, and in some of the cysts albuminous material which contained no separate psorosperms could be seen to be in process of subdivision into these spores; thus material histologically indistinguishable from colloid-matter was proved to have been a syncytium of living parasites ripe for subdivision into spores. The spores in many places, both of the ureter and the kidney, were found to have passed into the connective tissue and there to be associated with inflammatory changes.

I have been able to find small reticular parasites close to the nuclei of epithelial cells of the cysts of the ureter, but at the neck of the bladder where numerous cysts were present with much inflammatory infiltration, the only change in the epithelial cells seemed to consist in an increase of granularity of the cell-protoplasm, and this may be the earliest evidence of infection. The smaller parasites were highly refracting and refused to take any stain save occasionally at a nuclear spot. The dense deeply staining protoplasm was assumed by the older parasites and again lost when the reticulation which accompanied spore-formation set in. The ripe parasites more closely resembled certain gregarinida than the coccidia of the rabbit's liver. L. Pfeiffer has kindly sent me sections of the kidney of a goose containing coccidia which differ distinctly from those of the case under consideration, in being much smaller, in possessing distinct capsules, and in other ways. In the goose's kidney as in the renal psorospermosis of man fusion-masses of parasites are formed. The biological knowledge of the sporozoa is at the present time so imperfect that it is hazardous to propose the classification of any new form of these parasites. Time after time what have appeared to be distinct species have proved to be phases of previously classified sporozoa.

The importance of these cases of psorospermosis of the urinary tract of man is great. They teach that sporozoa having affinities both with the gregarinida and the coccididea occur in man causing not only formation of cysts, but infiltration of connective tissues and inflammatory changes. Moreover, I have found in the cysts of the ureter evidence of new formation of glandular elements or in other words an adenomatous process.

Further these psorosperms of the ureter are in all the varied stages of their life-history repeated in every one of the squamous epitheliomata and cancers of the uterine cervix which I have examined for them; and other cancers contain bodies which though not in every phase absolutely identical in structure with those of this case, yet afford as much evidence of being closely allied sporozoa.

The case proves beyond question that there exist as pathogenic parasites in man, sporozoa which multiply neither by hard-shelled lasting spores, nor by sickle-shaped swarm-spores, but by minute granular amœboid swarm-spores which are at first devoid of a nucleus having their chromatin diffused throughout their substance. All the reasons which avail to establish the nature of these psorosperms of the ureter apply equally to the parasites of cancer.

The chief of these reasons are :—

1. At certain stages the parasites resemble recognised sporozoa. The intra-cellular stages being of especial importance in diagnosis.

2. At a certain stage, which elsewhere I have termed "ripe," they tend to assume a round or oval form, their protoplasm is dense and highly refracting and stains deeply with carmine, acid fuchsine, &c. Their "nucleus" often does not give the reaction of chromatin to acid hæmatoxylin.

3. Some of these bodies present structural features incompatible with anything save a state of perfect vitality. I may instance the presence of nuclear figures such as are represented in Pl. iv., fig. 2, Path. Soc. Trans. 1892.

4. All stages of swarm-spore formation can be observed.

Foa[60] at first unconvinced by the work of Podwyssoski[61] and others that parasites were present in cancer, found in some breast cancers cell-inclusions which he could only regard as sporozoa. The tissue was fixed in sublimate solutions hardened in alcohol and stained in hæmatoxylin. The bodies regarded as sporozoa by the

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60. Foa. "Centralblatt für Bakt." Aug. 9th, 1892.

61. Podwyssoski has completely answered Foa's objections. "Cenralt für Bakt," Oct. 19th, 1892.

author resemble that given lower down (fig. 22, 3). The same author, in a later article,[62] gives farther particulars of the parasites observed in nine cancers of the breast and one of the lung. The young parasites stain with hæmatoxylin, older ones have a nuclear body which only stains with eosine. This central body subdivides resulting in the formation of spores which penetrate the substance of fresh cancer cells.

M. A. Ruffer, in collaboration with J. H. Walker[63] and H. G. Plimmer,[64] has published three papers bearing on the subject. If the second paper is compared with the first a modification of the authors' views becomes apparent; for five of the drawings of the first paper are reproduced in colours in the second, and whilst in the former paper parts of the Figs. 4, 6, and 7, are said to represent parasites, the same parts in the second paper are given as representations of degenerated cells, &c. Therefore it will suffice to notice the two latter papers. The first of these is accompanied by thirty-seven drawings, twelve of which are by Metchnikoff. The second is accompanied by thirteen illustrations, which recall Podwyssoski's drawing Pl. i. Fig. 10, accompanying Sawtschenko's article. The bodies recognised by the authors as parasites are the same as some of those previously figured by Nils Sjöbring, Soudakewitch, and Foa. As will be seen below I agree with the authors in their interpretation, but I believe they show but one of many phases of the parasites demonstrable in cancer. The work is set forth with a histological skill which must leave a valuable impress on future investigations on the subject. The modification of the Ehrlich-Biondi stain introduced by Ruffer is of the greatest value for the demonstration of the parasites, but the authors in the work published in their third paper used hæmatoxylin and eosin, which I had previously found suitable for the study of the parasites.

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[62] Foa. "Gazz. degli Ospitale." Feb. 2nd, 1893.

[63] Ruffer and Walker. *Brit. Med. Journ.*, July 16th, 1892. *Journ. of Pathol.*, Oct., 1892.

[64] Ruffer and Plimmer. *Ibid.*, June, 1893.

The claims of the cell-inclusions described to be regarded as parasites are put forward in a manner unlikely to prove convincing. They are spoken of vaguely as "protozoa" without reference to any kind of protozoa previously known to the authors, indeed Metchnikoff's opinion which they quote to the effect that the bodies "have all the characteristics of coccidia," seems to be the only foundation of their view, and that being the case it is hard to understand why they have not recognised that if the bodies belong to the protozoa at all they are sporozoa. In the last paper the authors emphasise the fact that they have not been able to find any evidence of spore-formation. In this they stand practically alone. Their position may be explained by their mention of "real falciform bodies," implying that these are the only form of swarm-spore met with in sporozoa. This has been shown not to be the case, *e.g.*, by my case of psorospermosis of the urinary tract.

It is evident that the authors have not recognised the ripe and sporing parasites as I have described them in various cancers. They make no mention of parasites in sarcoma.

James Galloway[65] repeats observations of Malassez,

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[65] Galloway. Moreton Lecture. *Brit. Med. Journ.*, Feb. 4th, 1893.

Delépine, and others, on coccidium oviforme, and of Sjöbing, Soudakewitch, and others on cancer, and confirms a previous observation[66] of my own of parasites free in the connective-tissue of cancers.

W. W. Keen[67] reports a case of coccidial growth of the liver in man.

The "Morbid Growths Committee" of the Pathological Society[68] published *in extenso* what purports to be a report of my work.

At the first meeting of this committee I had to object to its constitution, and ask that a biologist might be added to their number because not one of the members of the committee showed any special knowledge of the sporozoa—the parasites in question. No biologist was added to the committee.

The report practically ignores the chief part of my work, namely, that on squamous epithelioma. A proceeding quite unjustifiable on any grounds whatever, as I should never have submitted my work to them save on the understanding that they would report fully upon the whole of it. The committee declined to allow me to demonstrate to them many of my sections of squamous epithelioma.

The committee, as I was informed by one of its members after the publication of the report, did not consult the manuscript of my communications to the Pathological Society. This manuscript was placed in the hands of the committee, and to it I referred them for a connected account of my work.

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[66] Report of Meeting of the Path. Soc. *Brit. Med. Journ.*, Dec. 24th, 1892.

[67] Keen. *Boston Med. and Surg. Journ.*, May 9th, 1893.

[68] *British Medical Journal*, May 20th, 1893.

If they had referred to the manuscript they would have seen that the bodies discussed in the Clauses 2 and 4 of their report were not mentioned in the manuscript and formed no part of the basis of my conclusions. The report is misleading in that it conveys the sense that I have put forward as facts what I gave out only as suggestions, which I had not allowed to influence my reasoning.

The following section on squamous epithelioma will suffice to show that it is not true that certain of my interpretations have for their foundation only my "bare assertion."

The committee "do not propose to discuss" my specimens of sarcoma, at the same time they make statements concerning them which are calculated to mislead. Whatever interpretation may be put on the appearances present in the sections they are clear and definite and not in any way "obscured."

The committee were able to recognise in some of my sections bodies similar to those previously demonstrated to them by Ruffer and Plimmer.

C. H. Cattle[69] describes and figures with great clearness cell-inclusions and other bodies in cancer of the breast. Their Figs. 1 and 3 resemble bodies described as parasites by Soudakewitch. Fig. 2 is an interesting variety of cell-inclusion which I have been able to find in sections of cancer of the breast.

Heneage Gibbs[70] on the strength of a comparison of sections of cancer with others of coccidial lesions of the rabbit's liver concludes that parasites are not present at any rate in the majority of "glandular" cancers. This result is open to the grave objection that the parasites of cancer are not identical with the coccidia of the rabbit's liver.

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[69] C. H. Cattle. *Brit. Med. Journ.*, July 22nd, 1893.

[70] Heneage Gibbs. *American Journ. of Med. Sci.*, July, 1893.

THE SPOROZOA OF CANCER.

The first cancer in which I recognised the presence of sporozoa was a squamous epithelioma sent to me

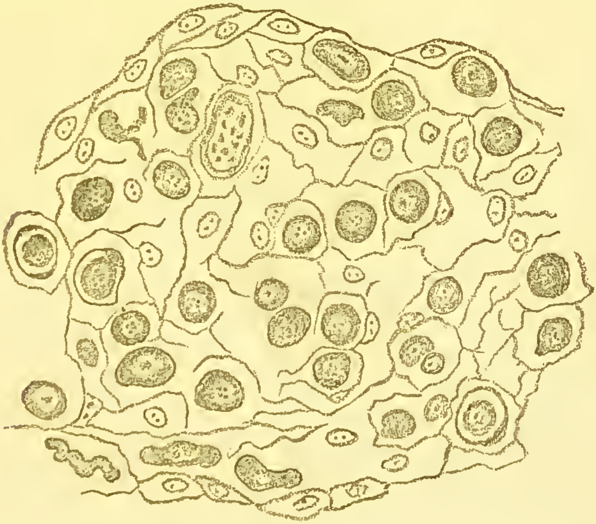

FIG. 5.—Part of a section of squamous epithelioma with numerous psorosperms within the keratinosed cells and free where these have broken down. Three of the parasites are closely surrounded by a second cell. One parasite is forming spores. The large parasites were stained dark-red, the spores blue. Biondi, Zeiss, D oc. 2.

for pathological diagnosis by Scanes Spicer who had removed it from the septum of the nose of a man, æt. 82. In reporting on this case in May, 1892, I wrote: "The growth is a squamous epithelioma and abounds in what must now be regarded as psorosperms." The structures which first attracted my attention in this case were round, oval, and irregularly shaped bodies some distinctly intracellular and having the same physical characters as the psorosperms of the ureter (Fig. 3). In the looser parts of the growth these bodies were present in great numbers. Dense highly-refracting, with clear nucleus, containing one or more nucleoli, they were more deeply coloured by carmine and fuchsine than any other cells in the growth. (See Fig. 5.)

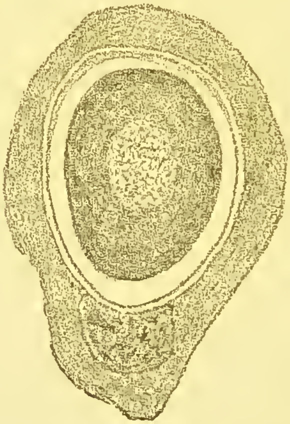

Fig. 6.—Epithelial cell containing an encapsuled parasite in which reticulation is commencing. Leitz, $^1/_{20}$ in.

These larger or ripe parasites were found chiefly in

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and about the cell-nests, but a few were present in the connective tissue spaces. Examination of sections stained with picrocarmine enabled the process of sporing to be traced with the same certainty as in the psorosperms of the ureter. In some parasites the nucleoli were absent from the nuclear region which was enlarged and occupied by a highly-refracting reticular structure (Fig. 6).

In many of the cells the parasite was entirely replaced by reticular spores, Fig. 8.

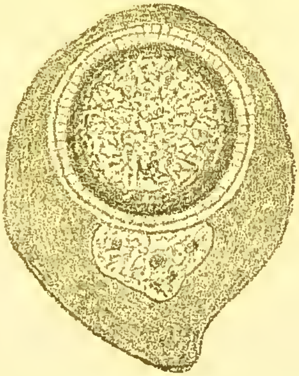

Fig. 7.—The reticulation of the parasite extends almost to the periphery and radial striæ pass from the parasite to and through the capsule. Leitz, $^1/_{20}$ in.

These bodies have all the characters of those I have described in the cysts of the ureter, and they were easily distinguished from epithelial cells and their spores from leucocytes. In sections treated with acid-hæmatoxylin, the appearance of the spore broods was in some instances of great interest.

Fig. 9[71] represents with its contents a capsule which was situated at the margin of a cell-nest, see Fig. 16. In another optical section the nucleus of the host-cell

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[71] This fig. was published in the *Brit. Med. Jour.*, Dec. 24, 1892.

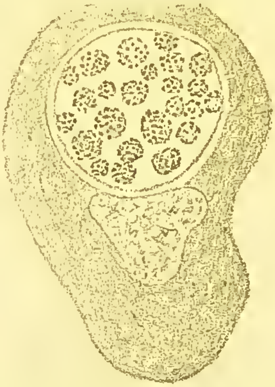

FIG. 8.—Epithelial cell in which the parasite has broken up into spores (2·6 μ in diam.) Picro-carmine and glycerine. Leitz, $^1/_{20}$ in.

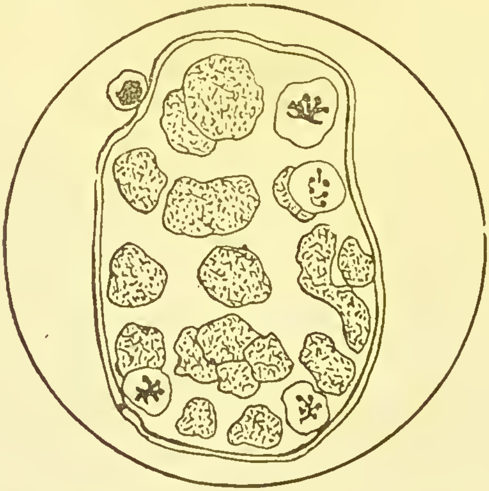

FIG. 9.—An encapsuled parasite in the course of subdivision. The reticulum of the non-nucleated bodies stained purple with hæmatoxylin. Leitz, $^1/_{20}$ in.

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was to be seen adherent to the capsule. The capsule is filled with minute corpuscles, some delicately reticular and staining faintly purple throughout, others were clearer and possessing nuclei composed of beautiful radiating bars of chromatin. The reticular bodies are neither epithelial cells nor leucocytes, they are similar to the reticular spores in Fig. 8, the same as the reticular spores of Wickham (see Fig. 1) which Borrel has assumed to be products of degeneration, but which, from their mode of origin, structure, and staining reactions, are certainly cells in full vital activity. The nucleated bodies differ from the others in having their chromatin condensed to a nucleus instead of being diffused through the whole of the cell. In the neighbourhood of such cysts, and sometimes occupying the place of the bodies just referred to, are corpuscles similar to that adhering to the outer surface of the capsule in Fig. 9. Bright minute corpuscles 2-3 μ, identical with the spores as figured by Soudakewitch (see Fig. 1). It is probable they result from the farther subdivision of such bodies as represented within the capsule of Figs. 9 and 10, which in their turn result

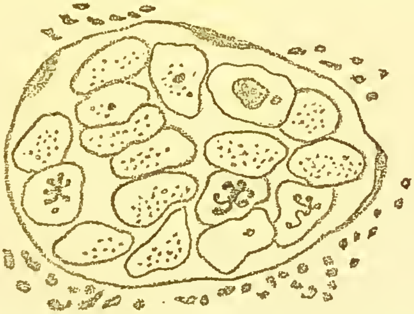

Fig. 10.—Waldeyer-cell-sac filled with parasites and surrounded by swarm-spores. After L. Pfeiffer.

from the subdivision of ripe parasites in which I have in many sections traced their origin. In his latest work, L. Pfeiffer[72] figures in a fibre of a muscle infiltrated by cancer a cluster of bodies almost identical with those of Fig. 9, and takes the same view of them as I do. L. Pfeiffer has given reasons for believing that the infiltrating cells are comprised almost entirely of parasites.

In everyone of thirty different examples of squamous epithelioma, I have been able to find these bodies which have all the characters of the psorosperms of the urinary tract as described by me, but the epithelioma of the septum of the nose showed them, perhaps, more plainly than any of the others. The reasons for this are, I believe, to be found in the fact that the growth was of mushroom type, hence the pressure exerted on the cells and parasites was little, and so the shape of the parasites was but little modified, and moreover, the epithelial cells in this growth were large and the parasites were large in proportion. L. Pfeiffer has shown that in very many instances the size attained by sporozoa is in proportion to that of the host-cell. The following description applies to the parasites of all the squamous epitheliomata I have examined, thirty-one in number. The earliest intracellular parasites consist of non-nucleated or nucleated particles apparently situated within the nucleus, as seen in the drawings of Sjöbring and Soudakewitch (Figs. 1 and 2). Parasites of rather larger dimensions lie in cavities close to the nucleus of the host-cell, which is indented. As the material was hardened in Müller's fluid and spirit, these young parasites which were most of them round with a central nuclear spot had shrunk a little away from the walls of the cavity containing them. Older parasites were for the most part distinctly encapsuled and presented appearances which excluded the possibility of their being anything but active and healthy cells. In Fig. 11 five parasites are represented, the host-cells being omitted. They serve to illustrate the variety of nuclei found in the parasites. In the lower one to the right the nuclei are multiplying by division.

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[72] L. Pfeiffer, "Untersuch. üb. den Krebs," 1893.

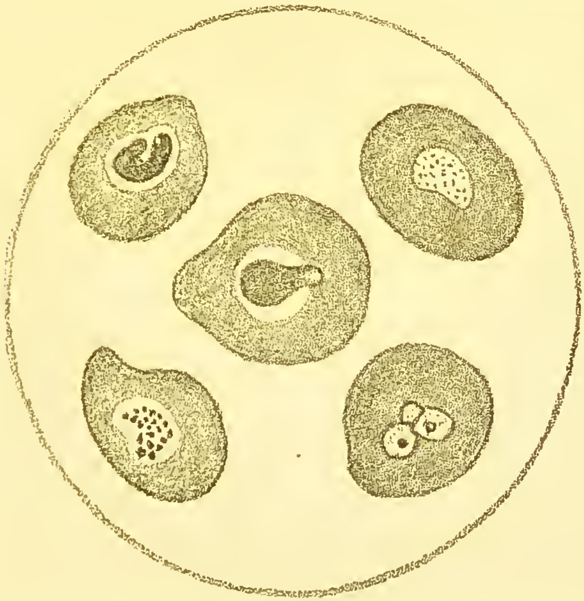

Fig. 11.—Five psorosperms from a section of the epithelioma of the septum of the nose. Hæmatoxylin, Leitz $^1/_{20}$ in.

Comparison with other parasites leads me to the conclusion that this is one mode of reproduction, the nuclei continue to divide whilst the protoplasm diminishes until only a cluster of minute cells remain. This I believe, to be one mode of origin of Virchow's "nests," the formation of which is compared to that of pollen grains ; these are Sjöbring's "spores." (See Fig. 1.) These same "nests" sometimes result from the abstriction of small cellules from the periphery of a parasite as will be shown in connection with cancer of the breast, or again from the direct division of the parasites.

Many giant nuclear figures such as I have described in the psorosperms of the ureter, "Trans. Path. Soc.," 1892, Pl. iv, Fig. 2, were present in these psorosperms, also the more familiar "giant mitoses" such as the one represented in Fig. 12.

After careful comparison of sections of many cancers I have come to the conclusion that these giant mitoses, in many cases at least, belong to parasites. Secondary growths of squamous epithelioma in glands show this most distinctly, for in a series of sections dense ripe psorosperms and encapsuled cell-inclusions with giant mitoses can be seen to occupy corresponding positions, namely, the central parts of the epithelial columns, and both can be seen to be breaking up into swarm-spores.

Many interesting features are brought out by the Ehrlich-Biondi triple stain. Some encapsuled parasites present a nucleolus deeply stained with fuchsine, outside that a deep blue zone, the two being separated by an unstained interval, and most externally a faint ky-blue zone. These appearances are similar to those represented by Metchnikoff.[73] In such parasites between the two blue zones the nuclear membrane sometimes appears, and it may be connected by radial bars with the cuticular layer of the cell as is shown in Fig. 13. In this cell there was a faint but distinct radiation

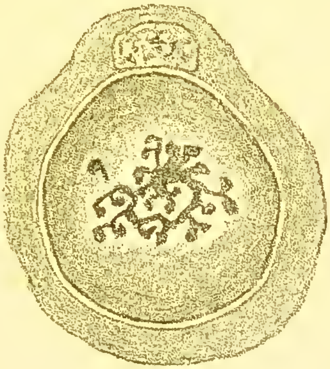

FIG. 12.—Intra-cellular psorosperm with giant mitoses. Leitz, $^1/_{20}$ in.

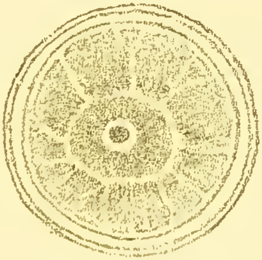

FIG. 13.—Encapsuled parasite, nucleolus was red, the rest of the body of the parasite was blue with radial striæ. The nuclear membrane remains and is joined by radial bars to the cell membrane. Leitz, $^1/_{20}$ in. Biondi.

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[73] Ruffer and Walker. *Journal of Pathology*, October, 1872.

of blue lines passing from the white zone outside the nucleolus and again from the outer surface of the nuclear membrane.

This phase of the parasite seems thus to be due to a generalisation of chromatin preparatory to subdivision.

In connection with the psorosperms of the rabbit's liver I have shown that some of the parasites had almost the morphological significance of nuclei, and I shall show that the same holds for the parasites of cancer at certain stages. The parasites shown in Fig. 5 and elsewhere react quite differently to this stain; they take a red or red-brown colour and their nuclear particles stain in the same manner as the rest of the cell, such a body faintly stained is, I believe, represented by Metchnikoff.[74]

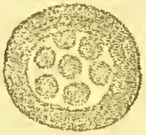

Fig. 14.—Ripe parasite with seven spores. Zeiss, F. Biondi: Spores blue, remainder red.

The youngest intra-cellular parasites also frequently stain blue throughout with this reagent.

These differences of staining reaction point to vital processes in the parasitic cells and they gain some explanation by studying the process of sporing. In many of the ripe psorosperms this occurs in a most regular and beautiful manner. In Fig. 14 is represented a parasite in which spores are forming. The seven round bodies in the centre were stained a bright blue with the Biondi reagent whilst the remaining peripheral part of the parasite was dark red. When cleared and mounted in balsam the reticular structure of the spores is but faintly seen.

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[74] Ruffer and Walker, loc. cit., pl. xiv, Fig. 9.

In sections mounted in glycerine the reticular structure is better marked, and when stained in iodine green the nodal points of the reticulum stand out with great clearness. (See Fig. 15.)

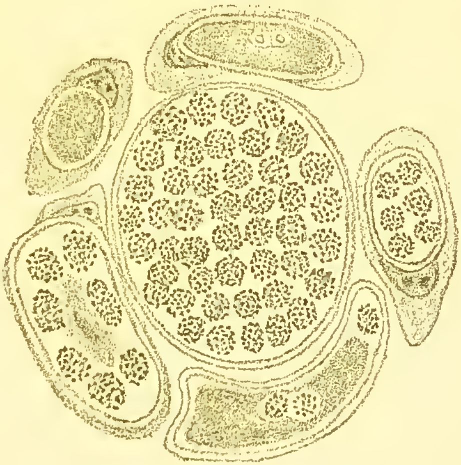

FIG. 15.—In the centre, in optical section, an encapsuled parasite completely divided into spores, below a parasite with two spores formed in the centre and one at the periphery, below and to the left an intra-cellular parasite with peripheral sporing. Spores deeply, the rest faintly stained with iodine green. Mounted in glycerine. Leitz, $^1/_{20}$ in.

In some of the cell-nests were large numbers of free parasites altered in form by mutual pressure and giving the same staining reactions as the intra-cellular ripe parasites, *e.g.*, dark red with carmine or Biondi. These parasites were distinguishable from masses of keratin by their staining deeply with carmine,[74a] and from the fact that many of them were observed to have gone through the same processes as the intra-cellular parasites, some having undergone complete reticulation, and by fusion having formed large plasmodial masses which in some instances were observed to be breaking up into reticular spores. (See Fig. 16.)

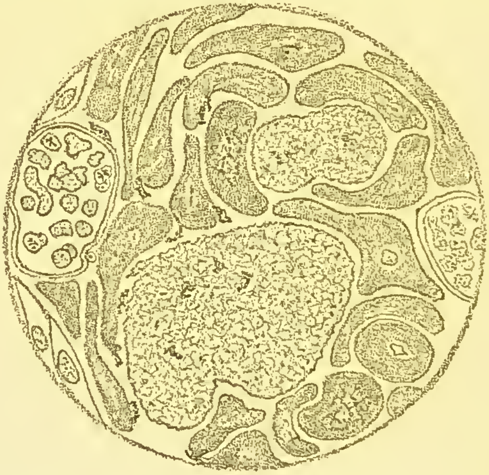

Fig. 16.—To the left are three epithelial cells and the capsule filled with spores which is represented in Fig. 9. Numbers of ripe psorosperms and two reticular masses are also shown. There are some free bodies resembling irregular nuclei. Acid hæmatoxylin. Zeiss, F.

[74a] This distinction only applies to cells completely cornified and not to such as contain kerato-hyalin.

From the foregoing description, it will be seen that the type of sporing is the same in squamous epithelioma as in the psorosperms of the ureter. (Figs. 3 and 4.) In many sections large sacs filled with regularly rounded spores, averaging 5 μ in diameter, are abundant. The spores are distinctly granular (see Figs. 8, 14, and 15) or reticular (see Figs. 8, 9, and the fourth of Wickham's drawings in Fig. 1). The granules and nodal points of the reticula stain with carmine and logwood. The parasites, however, are not restricted to this mode of reproduction. In Figs. 19 and 20 nucleated and non-nucleated daughter-parasites are seen side by side. Many encapsuled cell-inclusions can be found completely subdivided into minute bodies, some of which resemble the reticular corpuscles in Fig. 9. Others are like them, but stain more intensely with acid hæmatoxylin and show signs of subdividing into several smaller bodies which closely simulate the nuclei of leucocytes ; others again are clearer with distinct nuclei. This last variety also increase in numbers by division, which in them is effected by a mode of karyokinesis. Such collections studied in encapsuled intracellular spaces or isolated in teased specimens are easy to distinguish from leucocytes, but where similar bodies arise from the subdivision of ripe parasites lying free they may be indistinguishable from wandering leucocytes. This fact is sufficient to make the study of phagocytosis in cancers one of extreme difficulty. It is probable that this process occurs especially in slow-growing and atrophic cancers. Still, for the present, figures such as those by Metchnikoff[75] must be received with caution, because

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75. Ruffer and Walker. *Journal of Pathology*, Oct., 1892.

quite similar appearances result from the subdivision of the parasites.

The reaction of the spores to the Biondi reagent is of interest. The granular spores are usually stained purple or green, but they may be red or orange. The nucleated spores are sometimes indistinguishable from leucocytes, save by their having a greater refracting power and a more granular appearance. The study of many hundreds of sections and teasings of squamous epithelioma enables me to affirm that the well-known appearance in cell-nests represented in Fig. 17 is the

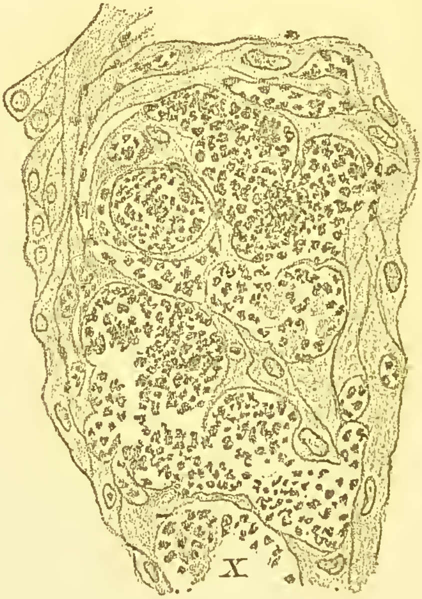

Fig. 17.—A cell-nest in which the transformation of the ripe parasites into spores is almost complete. Only portions of the original parasites remain, as to the left of "X."

result mainly of subdivision of ripe parasites, and not, as is generally believed, due to the immigration of leucocytes, which have digested the keratinosed cells.

In many of the cell-nests, the ripe parasites are massed together and in places fused together just as in the cysts of the ureter. The liberation of spores from these fusion masses could be traced in many sections, as could also the fragmentation of the reticular bodies into the spores. When carefully examined such parts afford sufficient evidence that these phenomena are not due to degeneration of epithelial cells and immigration of leucocytes. The accompanying figure reproduces a photograph which was kindly taken from a safranine-stained section of the epithelioma of the nasal septum by my friend and colleague, E. W. Roughton. In the microscopic field here represented, I could not find a single leucocyte. The minute dark bodies are delicately reticular, and are stained throughout with safranine showing no differentiation into nucleus and cell-body. They show evidences of further subdivision and are of varying size. Many of them had wandered among the surrounding epithelial cells. These bodies have been recognised by Nœggerath as living particles resulting from the subdivision of cancer-cells. They are generally regarded as leucocytes. In some cancers leucocytes appear to be admixed with them.

In all the thirty-one squamous epitheliomata I examined, the passage of the swarm-spores into the connective tissue could be traced. Fig. 18 shows a portion of a section of a cancer of the lower lip which had been hardened in a mixture of Müller's fluid and spirit. The amœboid spores were readily recognisable by their high refracting power, and from the fact that only a few of them possessed nuclei.

In all the squamous epitheliomata the effects of this invasion on the part of the parasites were plainly seen. Rows of epithelial cells are separated and thus the number of points of epithelial ingrowth are multiplied. A considerable amount of inflammation is caused by the invasion of the vascular tissues. This repeated in a cyclic manner is sufficient to account for a certain

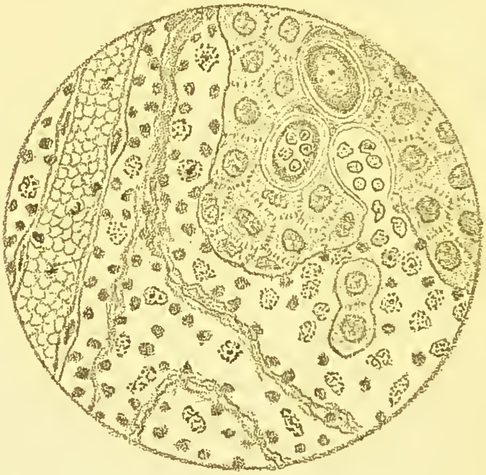

Fig. 18.—Cancer of the lower lip. In the epithelial part of the section are two encapsuled parasites, the upper one is clearing in the centre (reticulation) preparatory to spore-formation. In the lower one, five reticular spores have formed. One capsule has ruptured and the amœboid parasites are escaping into the connective tissue which contains many granular spores and leucocytes of which only the nuclei are shown. Two epithelial cells are detached. Zeiss, "F," oc. 2.

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amount of new-growth of the same kind as is seen in inflammatory papillomata. The resistance to epithelial ingrowth is thus removed by inflammatory softening of the tissues and subsequently checked by organisation of inflammatory tissue into vascular tissue. Study of sections of cancer of the tongue, &c., shows that of these invading amœboid parasites some enter new epithelial cells, others infiltrate muscle-cells. The former in the course of growth accumulate about the central parts of the in-dipping columns of epithelial cells and ripen to break up again into spores and renew the destructive process.

With regard to the capsules, it may be observed that some large intracellular parasites are devoid of any capsule, the thinned-out remains of the host-cell bounding the space in which they lie. In other cases the capsule is seen to be a double structure consisting of two concentric capsules which are joined together by radial bars. (Fig. 19.)

In squamous epithelioma cell-inclusions with radial striation of their peripheral protoplasm are common. I have observed in them the same process of reticulation that is met with in indubitable parasites and is not present in any known tissue-cells. I therefore conclude that they also are parasites.

In every kind of cancer variations from the round or oval form are common in the intracellular parasites. Sjöbring and Podwyssoski[76] have figured such forms in the dense or ripe stage which I have been able to identify as parasitic by comparing similar forms in the psorospermial cysts of the ureter. Figure 36 shows such a condition. There the parasite with Biondi stained deep red save at the nucleus which was green, showed a distinct longitudinal striation, which was also well-marked in many of the free parasites.

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[76] Sawtschenko, "Centralbl. für Bakt.," July 5, 1892, Figs. 2, 3, 5, &c.

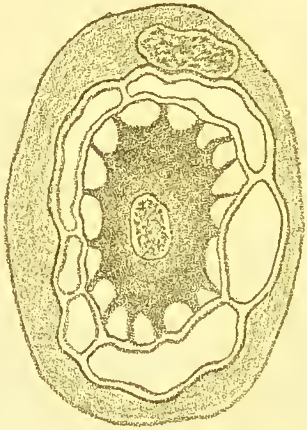

Fig. 19.—Intracellular parasite with a double capsule and peripheral processes similar to those figured by Wickham and Soudakewitch (see Fig. 1). Gentian-violet. Leitz, $^1/_{20}$ in.

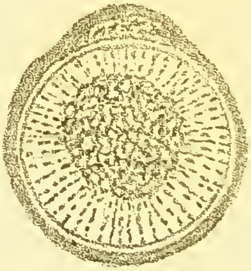

Fig. 20.—Intracellular parasite reticulated centrally, radially striated at the periphery. Leitz, $^1/_{20}$ in.

Such forms of cell-inclusions account for the presence of irregular intracellular bodies which have the same staining reactions as the ripe parasites without having any distinctive structure.

"CELLS OF ENDOGENOUS ORIGIN."

It is precisely in sections which best show Virchow's "cells of endogenous formation" that these are seen to have characters totally unlike any known tissue-cells, and a structure identical with that of certain phases of known coccidia. The labours of Flemming have made familiar the stages of the life-history of tissue-cells and have given a basis for comparison.

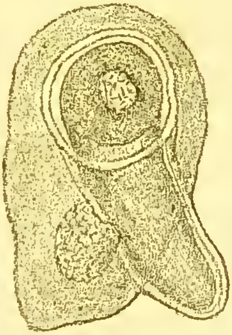

FIG. 21.—Intracellular psorosperm which has an elongated form and has caused a bulging of the host-cell. Leitz, $^1/_{20}$ in.

In Fig. 21 the frequent appearance of a "peripheral granule layer" in encapsuled cell-inclusions which possess no nucleus but give a faint nuclear reaction throughout is characteristic of and, I believe, peculiar to sporozoa. The body opposite "7" in the same figure shows the process of central reticulation mentioned earlier in this work (see Fig. 7, &c.). The contrast between the dense deeply-staining outer portion of this body and the bright almost unstained reticulum of the central part is unknown in tissue-cells. When we look back to the first description of "endogenous cells" we find that there is something of hypothesis and assumption at the very basis of the great patholo-

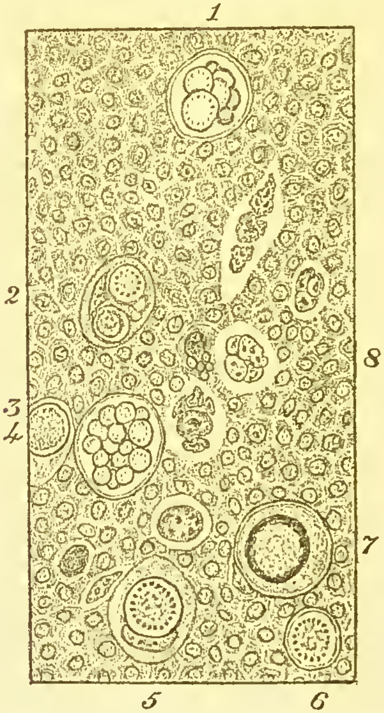

FIG. 22.—Section of a cancer of the penis. At 1 and 4, clusters of parasites, showing the "peripheral granule layer." 2, 3, 5, 6, separate parasites, showing similar characters. 7, ripe parasite undergoing reticulation. In the middle, opposite 8, group of minute parasites in a cell. Also numerous free bodies, possibly parasites. Zeiss, F.

gist's interpretation : " vielleicht ein auffallende Kern." Nœggerath has endeavoured to show how the cell-inclusions arise from nuclei. They do not arise by karyokinesis. From the first whilst yet within the nucleus these bodies (see Fig. 1) contrast strongly with the nucleus which contains them. True the contrast is not greater than that which exists between a fœtal red-blood corpuscle and the connective tissue cell in which it has an endogenous origin. But in cancer the endogenous cells have the characters, and, as may easily be seen in many sections, the life-history of sporozoa.

In a series of thirty sections cut from a portion of this cancer of the penis, appearances similar to those represented in Fig. 22 were present in only two sections, in the others the formation of swarm-spores from ripe parasites collected in and about the cell-nests was abundantly present. Although the peripheral particles are granular and not in the form of rods, I think the difference between these phases of the parasites is comparable to that which I have shown to exist between those of the coccidium oviforme of the rabbit's liver.[77]

The oval coccidium (dauerform of L. Pfeiffer) breaks up into swarm-spores, see Fig. 23 to the left, and corresponds with the large ripe psorosperm of the cell-nest region. The larger modifications of the C. oviforme (Fig. 23 to the right and Fig. 25) probably correspond to the parasites 1-6 of Fig. 22. They give evidence of increasing in numbers by simple division.

Cell-inclusions with a well-marked peripheral granule layer are not peculiar to squamous epithelioma. Soudakewitch has described similar[78] forms in cancers of the breast and in a cancer of the pancreas.[79] Ruffer would appear not to have recognised this phase of the parasites.

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[77] Jackson Clarke. *Med. Press*, Aug 30th, 1893, p. 209.

It does not avail to say that these parasites present in great numbers and giving evidence of rapid pullulation in squamous epithelioma are accidental epi-

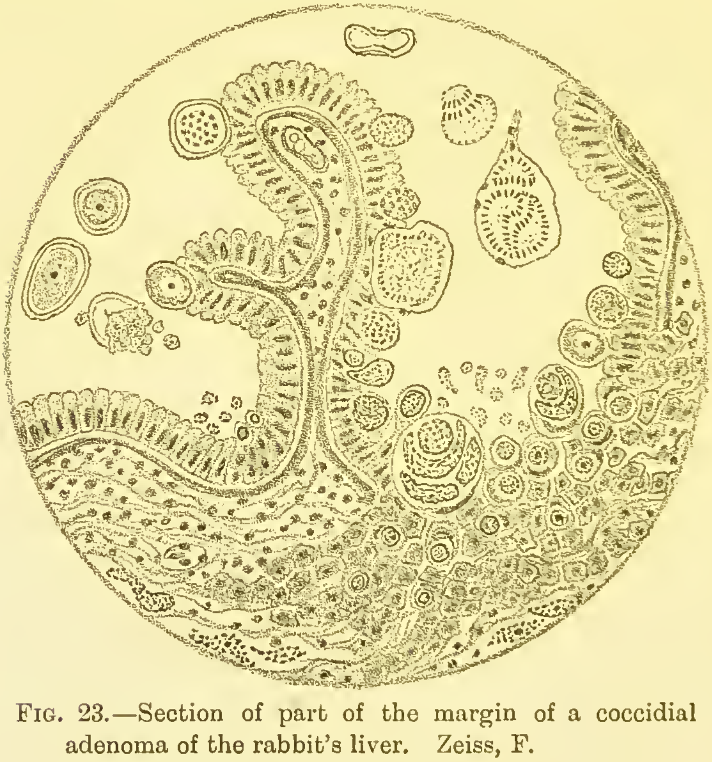

Fig. 23.—Section of part of the margin of a coccidial adenoma of the rabbit's liver. Zeiss, F.

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[78] Soudakewitch. Ann. de l'Institute Pasteur, 1892, vol. vi p. 156, pl. v, fig. 26.

[79] *Ibid*, p. 516, pl. viii, figs. 6, 11, 13, and pl. xi, fig. 9.

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phenomena. The bodies here described as parasites comprise parts of the growth long recognised as essential. They constitute the greater part of the cell-nests, and the actively invading elements which give the characteristic malignancy to the growths; at least some of them are included in Virchow's "cells of endogenous origin," and I believe an old and now (I think unjustly) discarded observation of Rokitansky's what he termed the intracellular formation of cysts, receives its explanation and its vindication. The diagram from Rokitansky's essay reproduced by Paget[80] showing clusters of minute daughter-cells I believe to refer to the sub-division of parasites. Indeed the sub-division of intracellular parasites such as has been shown in Fig. 8 results in the formation of intracellular cysts.

If the bodies are rightly described as parasites histological study is in my opinion sufficient to show that the malignant character of the growths is due to the invasion of the normal tissues abutting on the growths, and of the lymphatic vessels and glands. If it be conceded that this view of these bodies is correct then there is as much reason for considering them to be the cause of cancer as there is for considering the coccidium oviforme to be the cause of the cystadenomata of the rabbits' liver.

The importance of this form of epithelioma is increased by the fact that the only cancers (out of 150 cases) in which Soudakewitch could not find sporozoa were six cases of squamous epithelioma. This is explained by the fact that this author has not recognised all the forms of the parasites present in these growths, namely, some of the forms described by L. Pfeiffer, Wickham, and myself.

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[80] Paget. "Lectures on Surgical Pathology," 1876, p. 717.

CANCER OF THE CERVIX UTERI.

The ripe parasites are most easily recognised as typical sporozoa in sections of cancers of the uterine cervix, and in the secondary growths in the bladder and pelvic glands. Sections examined with a high power of the microscope show in the newly-formed tubules of the growth oval bodies for the most part lying within distinct capsules some, however, being naked, and having in the main the features given in my description of the psorosperms of the ureter. In these cancers it is easy to see that the capsules where they at present belong to the parasites. The process of swarm sporing is also easy to trace. In Fig. 28, 7, and 8 are represented two parasites, the capsules being omitted. From one parasite clear highly refracting granular amœboid spores (4-5 μ) are separating at the periphery, giving rise to an appearance somewhat resembling the sporing stage of Drepanidium ranarum (Ray Lankester).

In these cancers, too, the passage of the amœboid spores into the connective tissue is plainly visible. The spores contrast with the ripe parasites. The former remain bright and clear in sections stained with picrocarmine and acid hæmatoxylin, whilst the fully developed parasites are deeply coloured by the same reagents. In the connective tissue many of the amœboid parasites fuse together, forming masses o bioplasm, and where they cluster upon a fibrous bundle the latter is digested by this bioplasm, so that a strand of fibres deeply stained with carmine is interrupted by the parasitic mass which lies unstained and bounded by sinuous borders in the place of the digested tissue. In these cancers also the intra-cellular stages of the parasite can be traced.

Cancer of the Breast.

My observations are based on the study of twenty cases, two of which were colloid cancers. Some were recurrent growths. Wherever the glands were affected they were examined as well as the primary growths, and they contained in every case the same cell inclusions and free bodies I believe to be parasites.

The observations of Nils Sjöbring, Podwyssoski, Sawtschenko and Soudakewitch are easily to be verified in these growths, especially in sections of tissue fixed with osmic acid.

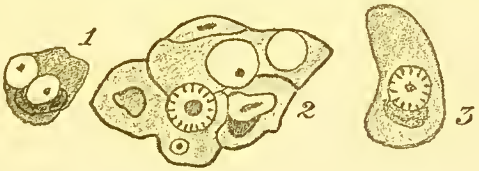

Fig. 24.—1. Two parasites close to the nucleus of a cell in a scirrhus of the breast. 2. A group of cancer cells containing five parasites, one of which contains peripheral bars of chromatin, from two of the others fine points of chromatin have been accidentally omitted (after Soudakewitch). 3. Cell of a cancer of the breast containing a single parasite with radial bars of chromatin (Flemming-Biondi). Zeiss, F.

Minute intranuclear parasites first figured by Nils Sjöbring (see Fig. 1) are with care easily discerned. In the solid epithelial parts of the growth, cell-inclusions, such as those represented in Fig. 24, abound.

Somewhat similar bodies occur in squamous epithelioma and can be traced in a continuous series, from the single intra-cellular parasite to the "nest" of minute cells, Fig. 22, 1 and 4. In many places cancer cells containing many such parasites are met with. These multiple cell-infections have been admirably described and portrayed by Podwyssoski, Soudakewitch, Foa, and others to whose writings I would refer for farther details, and although in the parts of cancers of the breast where these bodies are most abundant the formation of swarm-spores as it has been represented by Podwyssoski and Soudakewitch does occur, the process is not so marked as in squamous epithelioma and in cancers of the cervix uteri, and does not appear to account for all the cell-inclusions. This again points to a multiplication of the parasites by division. A mode of multiplication by unequal division I have also observed in intra-cellular parasites. This is represented in Fig. 28, No. 1, where an encapsuled parasite of which the host cell is omitted, is seen to have given rise to four daughter-parasites by abstriction from its peripheral part. Such a process when complete would result in the formation of one of Virchow's encapsuled "nests" of cellules which are the same as Sjöbring's clusters of minute parasites. (See Fig. 1.)

L. Pfeiffer, amongst others, has recognised this double mode of reproduction of the sporozoa of cancers, and finding the only group of sporozoa in which a similar process has been recognised are those called amœbosporidia, by Schneider, has suggested that this group may be the nearest to the carcinozoa. I have for some months been studying the phase of the coccidium oviforme which, as mentioned on page 210, seems to me to throw important light on the matter.

In rabbits' livers which contain numerous large and actively growing lesions, side by side with ordinary forms are great numbers of parasites in a state of multiplication by direct division. Some of the stages in this process of subdivision of the coccidium oviforme are shown in Figs. 23 and 25.

The coccidia in this modification possess rod-like bars of chromatin which are placed perpendicularly at the surface, and thus the distinction between nucleus and protoplasm is not present, or in other words, in this modification the parasites appear to be wholly nuclear in constitution.

The process ends in the formation of minute round cells or still more minute bodies, spores, in which also the chromatin is diffused throughout the structure as is the case in some of the reticulated spores of the squamous epithelioma. The two processes of multiplication, viz., by swarm-spore formation and by direct division, merge one into the other. It may be said of these minute bodies or cells in which the chromatin is diffused that they answer to Hæckel's primordial non-nucleated cell, or, since they stain with nuclear dyes and exist side by side with nucleated cells as part of

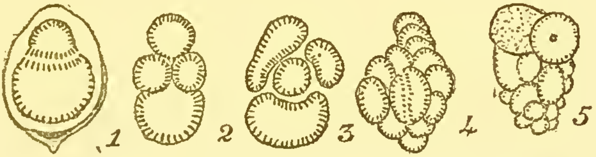

Fig. 25.—1, Intra-cellular parasite subdivided into two; 2-3, free coccidia divided into four; 4, cell cluster, division still proceeding; 5, cell cluster resulting from frequent subdivisions. Zeiss, F.

the same brood of daughter-parasites resulting from the sub-division of an encapsuled parasite (Figs. 9 and 10), that their chromatin is diffused throughout the cell substance. Indeed, it would be less incorrect to speak of them as wholly nuclear than as non-nucleated.

There is evidence in the nodules of the rabbit's liver of other modes of multiplication on the part of the parasites, but the one under consideration is here of especial interest since it explains the presence of clusters of parasites within single cells, and also for the peripheral radial bars met with in some of these inclusions (Fig. 24, 2 and 3).

The numbers and distribution of the parasites in the advancing zone of an ordinary cancer of the breast or its secondary deposits, when the evidence of multiplication by minute nucleated spores (as figured by Sjöbring, Podwyssoski, and Soudakewitch), and by direct division as described above are taken into account, suggest strongly a causal relationship between the parasites and the growth. Where a $^1/_{12}$ o.i. may scarce reveal the parasites a $^1/_{18}$, $^1/_{20}$, or $^1/_{25}$ o.i. shows them most strikingly and in great numbers.

The parasitic cell-inclusions may be clearly seen in fresh teasings. In a fresh preparation placed on the warm-stage I have been able to observe a slight amount of movement in a parasite which lay enclosed in a cell. The parasite was attached at one side only, and its nucleus was placed peripherally just opposite that of the host-cell. In ten minutes the parasite moved, so that its nucleus was no longer opposite that of the host-cell.

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In sections of healthy breasts taken during the period of lactation the ducts are filled with granular albuminous material containing fat drops and some chromatin particles. These appearances I have carefully considered before formulating the following conclusions :—The fat-globules, free fatty epithelial cells, &c., of normal lactation never, as far as I have been able to ascertain, arise in definitely encapsuled intracellular cells such as those represented in Figs. 26 and 27, nor does the granular albuminous material in the ducts of normal breasts assume the high-refracting appearance of the substance about to be mentioned as "bioplasm."

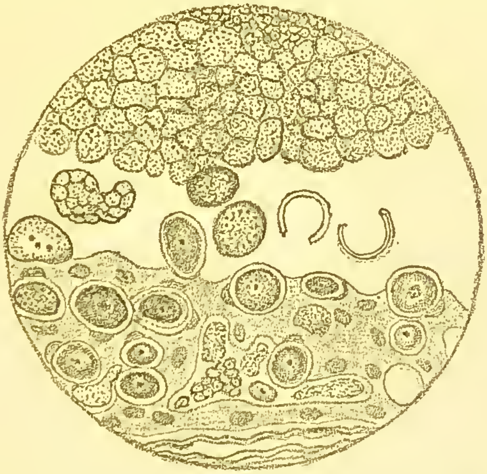

FIG. 26.—Section of a scirrhus mammæ. Numerous ripe psorosperms within epithelial cells, or free and reticulated in the cyst space, and some broken up into minute rounded or slightly angular particles. Two free capsules cut across. Müller's fluid. Picrocarmine and acid hæmatoxylin, glycerine mounting. Hartnack oc. 7, obj. 4.

Nils Sjöbring,[81] in his paper, has figured lying within cancer-cells dense deeply-staining bodies which he terms "sarcode," and which appear to be of the same nature as the irregular parasite represented in Fig. 21 and other figures of this work. This same dense texture of protoplasm marks the ripe parasites and fusion-masses of the psorosperms of the cysts of

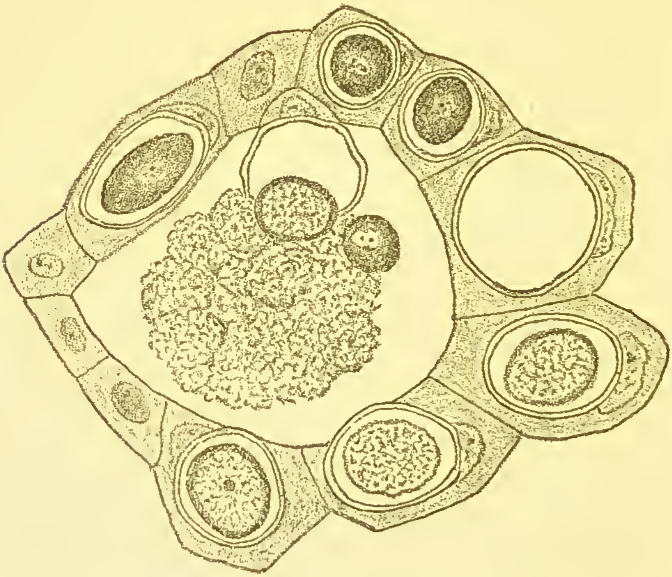

FIG. 27.—Section of scirrhus mammæ. Cyst-like space with the epithelial cells bounding it contain six ripe parasites, three of which are becoming reticulated. One reticulated parasite is escaping from the host-cell and joining a fusion-mass of reticulated parasites which constitutes the cyst contents. Flemming :—Biondi. Zeiss, F.

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[81] Nils Sjöbring. *Fortschritte der Medicin*, July 15th, 1890.

the ureter (Figs. 3 and 4). More definite cell-inclusions of similar texture are abundant at the margins of the cyst-like spaces of cancers of the breast. Such a form is delineated by Metchnikoff[82] as a parasite. This form of the parasite is met with chiefly in the older parts of cancers and in positions exactly corresponding to the cell-nests of squamous epithelioma, *e.g.*, the central parts of the epithelial tubes in cancers of the cervix uteri. In some sections they are especially numerous. They stain deeply with carmine, and when mounted in glycerine they have a much higher refractive index than the epithelial cells (see Figs. 26 and 27).

The nuclear particles of these bodies in many instances do not give the reaction of chromatin to acid hæmatoxylin, with the Biondi reagent they take a brownish colour in every part. In sections fixed in Flemming's fluid they come out more clearly than when Müller's fluid is used for hardening (see Fig. 27).

The interpretation of these bodies, which can be traced from encapsuled cell-inclusions to free bodies lying in cyst-like spaces, as parasitic, is supported by appearances represented in Fig. 28, 4 and 6. The latter is almost identical with a phase of Klossia represented by Max Wolters.[83] The nuclear membrane and the nucleoli remain, but nucleus and protoplasm alike are reticulated and refuse to be stained. A few similar cells are to be found free in the cyst spaces where, however, most of the escaped parasites are in the phase represented in Fig. 26, in which the nodal points of the reticulum are stained by nuclear dyes.

82. Ruffer and Walker. *Journal of Pathology*, Oct , 1892. Pl 14, fig. 9.
83. Max Wolters. "Archiv für Mikroskop. Anat." Vol. 37, pl. 8, fig. 8

The separation of swarm-spores from the parasites which have thus escaped into the cyst-like spaces is

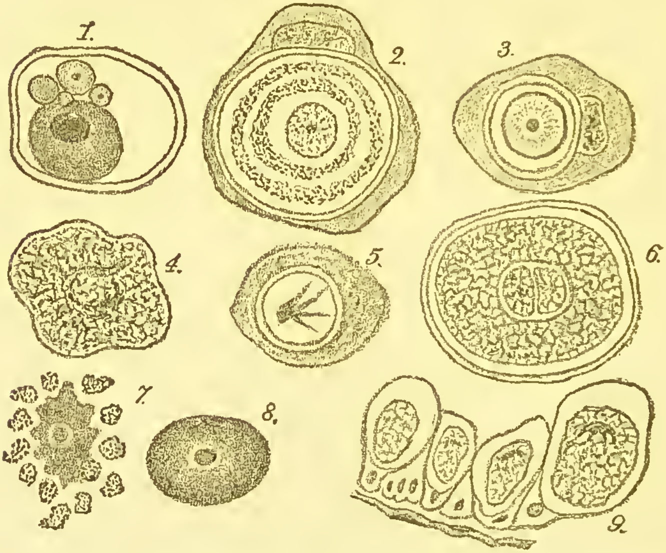

FIG. 28.—1. Encysted parasite multiplying by abstriction of peripheral particles, cancer of the breast. 2. Cell of a cancer of the breast containing three reticular parasites one within the other. 3. Cell of cancer of breast containing a young parasite. 4. Free reticulated body from cyst-like space of cancer of the breast. A trace of the nuclear membrane remains and a bar of chromatin which is placed chiefly outside the position of the nucleus. 5. Cell of a cancer of the breast containing a parasite, in the centre of which is a chromatin body with processes passing outwards. 6. Encapsuled reticular parasite from the margin of a cystic space, cancer of the breast. 7. Parasite sporing, cancer of the uterus. 8. Ripe psorosperm, cancer of the uterus. 9. Cells lining a cyst of an adenoma of the breast and containing reticular parasites.

not so easy to trace as it is in the forms of cancer already considered, the reticulation would, from analogy with other sporozoa signify a preparation for the process, and in some parts of sections of cancer of the breast at points where the cyst-contents come into direct contact with the connective tissue, the former can be seen to be continuous with a highly refracting material which extends into the connective tissue and terminates often with a looped outline about which separate particles of similar material are scattered. I believe the parasites which escape with degenerated epithelial cells and ruptured capsules into the cyst-spaces become reticulated, fuse together, and invade the connective tissue as a mass of bioplasm which breaks up into round granular spores resembling those described in the cysts of the ureter, squamous epithelioma, and cancers of the uterine cervix. It is, I think, possible to follow the fate of the bright bioplasm which extends along the previously healthy ducts of the gland. Of extremely high refracting power the reticular substance in many places can be seen to have broken up into spherical granular spores identical with one form of spore-formation in the rabbit's liver, Fig. 23, and with that already described in the sections on psorospermosis of the kidney, squamous epithelioma, and cancer of the uterine cervix.

The granules of these spores are stained by acid hæmatoxylin and iodine green. Their action on the connective tissue can easily be traced where they have wandered from the lumen of the tube. The immigration is marked by the accumulation of inflammatory cells, as in the squamous epithelioma. The study of a series of such areas shows that they form the starting point of new cancerous foci in which infected epithelial cells are seen. Such foci I have shown by dissection in cancerous breasts (St. Mary's Hospital Museum, No. 1470), and their general histological features have been described by Raymond Johnson and Cecil Beadles.

The bioplasm found in cancerous breasts resembles that figured in the gregarina-adenoma of the cat's lip.[84] Its resistance to stains (at most it stains faintly brown with the Biondi reagent), and its extremely high refractive index differentiate it from coagula of ordinary body fluids. It is often to be found extending along lymphatics. I have been able to find nothing exactly like it in normal breasts, whether in the resting or lactating phase.

Sometimes solid bougie-like processes of growth extend along the main milk-ducts.

The bodies such as that represented free to the left and again to the lower part of Fig. 26, subdivided into bright granular corpuscles, indicate, I believe, a more direct mode of sporing. Similar minute granular bodies are represented free in the cystic space in Fig. 26. Such bodies occur in the middle of the coccidium-cysts of the rabbit's liver.

Many of them may be found in almost every section of cancers of the breast, and particles similar to those of which they are composed are to be found within the protoplasm and nuclei of the surrounding epithelial cells (see Figs. 1 and 2). Sometimes they present curved rods of chromatin as figured by Sjöbring, Podwyssoski, and Sawtschenko.

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[84] Jackson Clarke, *Med. Press*, Aug. 23, 1893, p. 182.

There are numerous other forms assumed by cell-inclusions and free bodies in cancers of the breast which are neither normal nor degenerated tissue cells, but which may be sporozoa. Their description would occupy too much space to be detailed here.

Creighton has pointed out that in the breasts of cats affected with malignant disease a form of lactation occurs even apart from pregnancy. I have found this to hold true also of human breasts affected with cancer and sarcoma. Creighton describes in the lumen of the ducts of the breasts of lactating cats large brownish cells, and in the connective tissue great numbers of yellow epithelioid cells. The same bodies were met with in cancerous breasts. I have been able to observe the same phenomena in normal lactating breasts of women who have died within two weeks of delivery. Creighton traces the formation of cancer to a misdirection of physiological processes. The yellow epithelioid cells which throng the connective tissue spaces in some cases of malignant disease in cats, he suggests, are to be traced to the lining cells of ducts which become large and granular, leave the basement membrane and invade the connective tissue, a similar process being observed during involution.

In cancerous breasts great numbers of "Mastzellen" are to be found, and also corpuscles with large granular nuclei which are identical in structure with some cells occurring in the gregarina-adenoma of the cat's lip, and on the other hand they have affinities both with Mastzellen and epithelial cells. This portion of the histology of mammary cancers requires further investigation, and since it is not essential to the present question, I think it unnecessary to discuss it in detail.

There is no risk of confusing these various cell-forms with the cell-inclusions, &c., described above.

CANCERS OF OTHER KINDS.

In many other cancers, amongst which were a primary cancer of the kidney with its widespread secondary growths, a melanotic cancer of the skin, one of the ovary, one of the peritoneum (primary source not ascertained) and two rodent ulcers, I succeeded in finding cell-inclusions similar to those described above as parasites. Indeed, in no single instance out of over sixty cases where the tissue has been fresh and well-hardened, have I failed to find these bodies.

SPOROZOA IN ADENOMATA.

The most familiar cases of adenoma-formation admittedly due to sporozoa is to be found in the coccidial adenoma of the rabbit's liver. As before stated I had been able to trace in the case of psórospermosis of the ureter some new-formation of glandular elements, in other words, of an adenomatous process. I was also led to look for sporozoa in adenomata from other considerations. In rabbits the pathological effects of the same species of sporozoa vary according to the age of the individual attacked by them. In Silcock's case of psorospermosis inflammatory areas and papillomata were produced by the same infection. The sarcosporidia[85] sometimes appear to remain harmless within their host-cells, sometimes to cause progressive cyst-formation.

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[85] L. Pfeiffer "Protozoen," or for a brief sketch, Jackson Clarke, *Med. Press and Circ.*, Aug. 30th, 1893.

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The occurrence in a cat of an adenoma containing gregarinæ[86] is of interest both pathologically and biologically. I see no improbability in the supposition that the same species of sporozoa may cause in one individual a benign, in another a malignant, tumour. I have examined several adenomata and cutaneous papillomata for the parasites, and in the former class of tumours I have succeeded in finding parasites quite similar to those already described in cancer. This was especially the case in a small adenomatous patch in the uterus of a patient who had also two ovarian adenomata.

In a rapidly growing cystic adenoma of the breast, cell-inclusions, some of which are represented in Fig. 28, 9., were present, and also free forms on which, from the sum of the appearances, I came to put the same interpretation. In several other adenomata (testis, kidney, &c.) I found similar bodies.

Sporozoa present in Sarcomata.

The late W. B. Hadden[87] recorded a case of multiple sarcoma. The specimens from this case were referred to the Morbid Growths Committee by whom the matter was again referred to the late T. S. Cobbold, who pronounced the lesions to be due to psorosperms. Wernicke[88] describes sporozoa found by Posada in lesions of mycosis fungoides. The growth affected the corium and the parasites were present chiefly in giant cells. They closely resemble cell-inclusions of cancers. Encapsuled coccidia-like bodies, some subdivided into daughter-cells, and clusters of minute parasites are represented in four photographs.

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[86] Jackson Clarke, *Med. Press and Circ.*, Aug. 23rd., 1893.

[87] W. B. Hadden, "Trans. Path. Soc," vol. xxxiv., p. 237.

[88] Wernicke, "Centralbl. für Bakt.," Dcc. 23th, 1892.

The most familiar of human diseases generally recognised as being due to sporozoa—the malarial fevers—have long accustomed the mind to the presence of these parasites in the blood, a formation of mesoblastic origin. Thus there was established a probability of cases of infection of some of the connective tissues. The study of obsolescent coccidial lesions of the rabbit's liver had familiarised me with sporozoa lying in inflammatory tissue consisting of giant and endothelioid cells, and devoid of some of their most familiar characters. Cell-inclusions have been long recognised in myeloid and other sarcomata. A nucleated cell lying within a space in a giant-cell of a myxosarcoma is delineated by Victor Babès.[89] Examination of such bodies with the higher powers of the microscope reveals a capsule bounding the space in which lies the included cell, and the latter are seen to have characters similar to some of the bodies already described as parasites in cancers. A cell of a myeloid sarcoma of the femur is represented in Fig. 29.

It contains two cavities bounded by capsules which stained red with the Biondi reagent. In one of the cavities is an entire cell containing three nuclear points (green), and part of another. In the other cavity is a single non-nucleated body adherent to the nucleus of the containing cell.

The bodies all alike possess a peculiarly coarse reticular structure and high refractive power, distinguishing them from the containing cell. They retain the orange of the stain. Careful examination of many sections established the fact that they were truly intra-cellular. Examination of many sections of normal developing bone failed to reveal any such cell-inclusions in the large osteoclasts and other cells. Adherent to many of the cells of sarcoma of bone are encapsuled reticulated structures (see Fig. 30), which are also, as far as I have been able to observe, absent in normal tissues, though smaller reticulated bodies devoid of capsules occasionally occur.

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[89] V. Babès, V. Zemssen's "Handbook of Skin Diseases." Art "Sarcoma.'

Examination of a round-celled sarcoma of the testis gave important results. In sections lightly stained with picro-carmine and mounted in balsam with the objective (Zeiss's "D") focussed just above the surface the middle part of each inter-vascular area was marked out by a bright belt of light. On focussing the surface of the sections these bright belts were found to consist of highly refracting globes and bodies identical in form with cell-inclusions described as para-

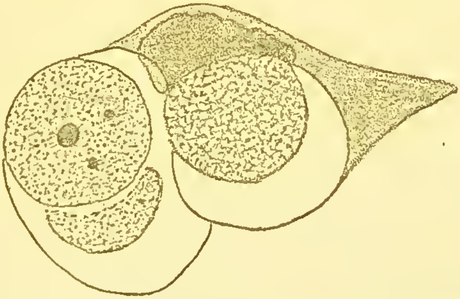

Fig. 29.—A sarcoma cell containing three parasites. Leitz, $^1/_{20}$. Zeiss, oc. 2.

sites in cancer. The smallest of these bodies were round or oval, highly refracting non-nucleated bodies lying in distinct spaces. Such a body lying between the two nuclei of a sarcoma-cell is shown in Fig. 31, No. 1. Its position and characters recall cell-inclusions met with in cancer, and raise the question whether mitotic nuclear division takes place in infected cells. One of Steinhaus's beautiful figures represents a cancer-cell in which regular mitosis is taking place a darkly stained oval body being present in the middle of the achromatic spindle between the two bundles of chromosomes. Soudakewitch also maintains that infected

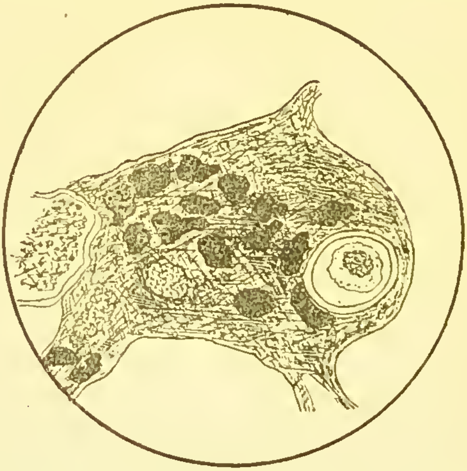

FIG. 30.[90]—A giant-cell of a myeloid sarcoma of the jaw containing an encapsuled parasite and having adherent to its left border an encapsuled reticulate body and two minute reticulated bodies in the cell-protoplasm.

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[90] This drawing was first published in a brief note in the *Brit. Med. Jour.*, Jan. 21st, 1893.

epithelial cells undergo indirect division, and the body under consideration suggests a similar process in sarcoma.

The inclusion (No. 2) within the cell to the left of the one just described in Fig. 31 with its radiating chromatin-rods is of a form frequently met with among sporozoa. Celli and Sanfelice[91] have described such bodies in the

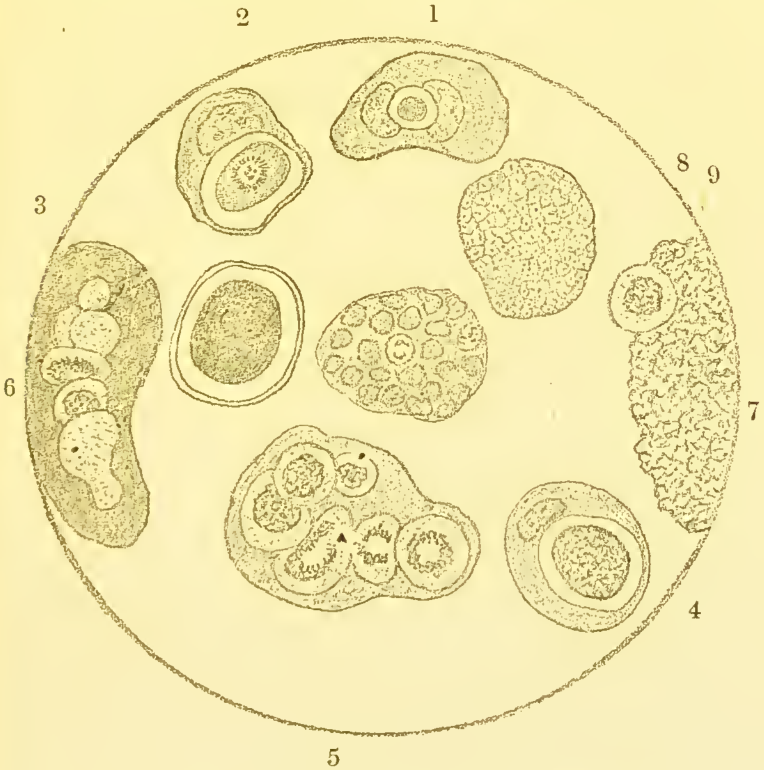

Fig. 31.—Various cell-forms in an intervascular area of a round-celled sarcoma of the testis. Zeiss, "F" oc. 2.

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[91] Celli and Sanfelice. "Fortschritte der Medecin," July 15th, 1891, pl. viii, figs. 31-38.

blood of persons suffering from quotidian ague and Soudakewitch[92] in cancers.

The encapsuled body (No. 3) to the left of the centre of the field had characters identical with some of the ripe psorosperms of squamous epithelioma. The nucleus had disappeared, leaving but a trace of its outermost part. Such bodies assume a dusky purple colour with acid hæmatoxylin, with carmine they stain deeply, but retain their transparency and show a high refractive power. With Biondi's reagent they stain orange red. Soudakewitch has figured a similar body in cancer.

Reticulation of the cell-inclusions as shown in the lower right-hand portion of the same figure (7) was easily seen, as were also large free reticula as shown to the right of the same figure. These reticulated bodies recalled those already described in cancer, and they may be of the same nature; cells subdivided into bright particles with central brighter points such as occur in cancer of the breast were also present (see No 8). The most important observation, to my mind, and which led me to conclude that these bodies were parasites, was that represented in three stages in the bodies 5, 6, 9, to the left, the lower, and the central parts of the field in Fig. 31. They lay for the most part free near the middle of the intervascular areas and by a process of internal subdivision, in large measure mitotic, result in the formation of collections of small (3-5 μ) highly refracting non-nucleated corpuscles. Such a process recalls sporing processes I have described in psorosperms of the ureter, and of squamous epitheliomata. The evidence of definite mitotic activity in the formation of these small bodies, I think excludes the possibility of their being due to degeneration or necrosis, although as in the active and obsolescent lesions of the rabbits' liver necrotic processes apparently exist side by side with intense biological activity on the part of the parasites. In rapidly growing myxo-sarcomata the phenomena I believe to be parasitic are most easily observed. Intracellular bodies, some small and dense, with or without nucleus, other larger and reticulated, all alike very highly refracting, are abundant. Free bodies of which I have been unable to find the homologues in fœtal connective tissue, characterised by a protoplasm similar to that of well-known sporozoa, that is to say, of peculiar density and correspondingly high refractive power, occur in great numbers in every part of the growth. In many places they are seen to be dividing by regular mitosis, but for the most part presenting irregular mitotic figures and giving evidence of intense vitality by their simultaneous subdivision into a great number of daughter cells. Some of these bodies are of great size (30 to 40 μ) with several central chromatin foci, generally hollow rhomboids with peripheral offshoots, and at the surface clusters of small rounded 2·6 μ particles, some with, some without, nuclei. These small bodies exactly resemble in every detail those I have represented in cancer of the breast (Fig. 28, 1) and appear to be the same as those delineated by Celli and Sanfelice in malarial blood (loc. sup. cit. pl. viii., Figs. 37 and 38). I think that these are parasites in active multiplication. They certainly correspond with no known phase of the life of tissue-cells.

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[92] Soudakewitch. "Centralbl. fur Bakt.," vol. xii, Nos. 14-15. 1893.

In several myeloid sarcomata I have observed similar evidence of subdivision in bodies which have the characters of sporozoa. In twenty sarcomata examined successively, similar bodies were present sometimes in great numbers in every part. Besides the forms already mentioned, the growths included melanotic sarcomas of various origin, and alveolar sarcomata of the skin. In only one case, an alveolar sarcoma of the muscles of the thigh, I failed to find cell-inclusions of the kind described above, and in these cases cells resembling sporozoa were present. If the interpretation I have ventured to put on these bodies is correct, there can be from their numbers and distribution in the growths, little doubt they are the cause of the disease. As Wernicke points out, the presence of sporozoa within giant-cells of sarcomatous type present a striking similarity to tubercular lesions, in the former sporozoa take the place of bacilli in the latter.

REVIEW OF OBJECTIONS TO THE VIEWS ADVOCATED ABOVE.

In considering the objections which have been brought against the interpretation of certain bodies in cancer as sporozoa, the first to be met is that bodies similar to some of the forms are encountered in normal tissues. In this connection it must be remembered that the sporozoa are animal cells possessing a wide and varied range of form, and in certain phases are indistinguishable from other animal cells. Before, in any case, concluding that sporozoa are present, I have satisfied myself that bodies having the characters of known sporozoa are present in three distinct phases: the intracellular, the free, and the sporing. It is a mistake made by bacteriologists to expect sporozoa to assume one invariable form or as nearly invariable, as for example the tubercle bacillus. When a broader view is taken such objections as that of Nussbaum[93] to the "sarcode" of Nils Sjöbring being regarded as parasitic fall to the ground.

The most natural attitude for one to assume confronted for the first time with the question of the presence of certain forms of sporozoa in familiar pathological formations is to attribute certain phenomena to degeneration. Such was my own first feeling with regard to the psorosperms of the ureter, and it was only after months of close investigation that I was able to prove to my own satisfaction that the bodies were certainly parasitic. The structure and mode of formation of the spores of these psorosperms of the human ureter has not as far as I am aware been described before, the previous descriptions of such cases being few and very incomplete. And since it was this mode of sporing which I was readily able to recognise and demonstrate in squamous epithelioma, the remarks of those who have not observed the process must be so far discounted. Ramsay, Wright, and others have explained as kerato-hyaline certain cell-inclusions described by others as parasitic in cancer. In the examination of many sections of papillomata with a view of determining in them the presence or absence of sporozoa I have never observed any condition of kerato-hyaline at all likely to be mistaken for sporozoa ; the drops are never intranuclear, never placed in a distinct cavity which indent the nucleus of the cell and so differ from most of the cell-inclusions regarded as parasites. They are not nucleated and so are distinguishable from the larger intracellular "dauerform" of the parasites. They

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93. Nussbaum. Archiv. f. Mikr. Anat. B. xviii, 1830.

stain deeply with logwood or carmine and so are readily distinguishable from the smaller clear intracellular parasites. There is of course no question of bodies such as those represented in Figs. 22, &c., being kerato-hyaline. Boeck[94] and others regard Darier's "grains," which the latter author himself has described as containing particles which react to stains in a manner similar to kerato-hyaline, as imperfectly keratinosed cells. Not having had the opportunity of studying a case of the kind personally I cannot here give a decided opinion, but I would point out that it is not impossible that the food-material stored in the body of various sporozoa should differ according to the nature of the host-cell and where the latter normally secretes kerato-hyaline this substance may be present in the parasites.

Again the fact that the granules present in these bodies react to stains like kerato-hyaline does not prove that they are composed of that substance. Moreover the great weight of the opinions of Malassez and Balbiani is in favour of Darier's view.

With regard to the bodies assumed to be "keratinosed epithelium" by the Morbid Growths Committee of the Pathological Society in the report referred to above, I can speak with more certainty. Contemplation of the bodies submitted to the committee reveals a series of forms incompatible with anything save vital activity, which excludes the view that the bodies are keratinosed. Though in large horny cells the nucleus can usually be demonstrated it never gives evidence of vital activity as do the nuclear bodies of the structures referred to in the report. In cell-nests

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94. Boeck. Archiv, für Dermatol. u Syph., 1892.

of some squamous epitheliomata every stage of sporing as described above, may be seen with even greater distinctness than I observed it in the psorosperms of the ureter. The meaning of these forms is lost on observers unfamiliar with the study of the sporozoa.

Borrel[95] has studied such forms only in a most imperfect manner as far as can be judged from his descriptions and drawings. He based his conclusions on the examination of a few squamous epitheliomata and of a warty tubercular lesion of the skin. At first inclined to regard certain bodies met with in teasing and sections as sporozoa allied to klossia, this author ended by arriving at the opposite conclusion.

The considerations which led him to this conclusion may be briefly enumerated and answered. He was unable to observe any formation of spores in the suspected bodies removed from the growth and kept moist. This negative observation is of no value whatever, especially as many sporozoa such as those of ague have not been observed to multiply in similar conditions.

The author was able to confirm the descriptions given by Wickham of certain structures met with in squamous epithelioma, but he found there might be placed on the appearances interpretations other than those given by Wickham. The fact that not one of Borrel's figures corresponds at all nearly with any of the bodies most faithfully depicted by Wickham invalidates the conclusions arrived at. For instance the structures represented in Borrel's, Fig, 1, B, "Globes à réticulum réfringent" are too indefinite to form the basis of any argument; they have neither the definite reticular structure of the "spores" represented in Wickham's Fig. 6, pl. 1 (Fig. 1 of this work), nor do they correspond with any of the "spores" such as I have depicted in Figs. 4, 8, &c. Yet I believe Borrel's figure refers to a sporing parasite, for in sections of the kidneys of snails infected with klossia as well as in the cell-nests of squamous epitheliomata amongst well-defined and, to a practised eye, unmistakable stages of sporing are some less definite forms, the meaning of which is clear when they are seen with their surroundings, but which are without meaning when seen isolated. By teasing fragments of cancers I have succeeded in isolating both in epithelial cells, and free in well-defined capsules, collections of spores of all the kinds described and figured in my work. (See Figs. 8, 16, 17, &c.) In spite of the fact that one of the most striking structural characteristics of the sporozoa consists in the formation of bright reticula either as a preliminary to sporing, or as residual bodies, Borrel seeks to settle the nature of the structures described by Wickham by applying the term "reticular degeneration," which, however, does not cover the gound without the further hypothecation of a spontaneous generation of minute cells which are neither leucocytes nor epithelial cells but arise in a rearrangement of what Borrel terms degenerated particles. The bodies represented in Borrel's Figs. 4 and 5, the latter from a warty growth, are also too indefinite to require discussion. Veritable parasites of quite as indefinite structure do occur, but they are only to be known as parasites when seen in connection with more definite forms. Sporozoa are well-known to be able to cause papillomatous growths[96] and are to be expected in some warts, though at present I have failed to find them there. The cell-inclusions with radial processes represented by Borrel and regarded by him as included prickle-cells, occur also in cancers of the breast where prickle-cells are absent and where Soudakewitch[97] would seem to consider them to be sporozoa, and this is my own view.

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[95] A. Borrel. Archives de Médecine Exp., 1890, vol. ii, p. 786.

The bodies described as parasites by Soudakewitch have been objected to on the score that they are "cells of endogenous origin." It is certain that Virchow included such bodies in the group, but as I have shown there is every reason to believe them to be parasites and the question arises whether the bodies termed cells of endogenous origin in cancers may not all be parasites.

Some writers, such as M. A. Ruffer, admit both the presence of intra-cellular parasites and the process of endogenous cell-formation. It is to be regretted that they have not definitely stated which of Virchow's figures they consider to refer to the one, and which to the other process.

Nœggerrath also describes in cancer large dense cells which undergo fragmentation. The description leaves little doubt that it refers to the bodies I have termed ripe and sporing parasites. Nœggerrath also describes anastomosing processes of these cells and other processes extending into lymphatic spaces into which are discharged the particles into which the peculiar cells divide. These phenomena are present in sections of squamous epithelioma, and are, I believe, demonstrably due to the sporing of ripe parasites. They offer, as Nœggerrath suggests, an explanation of toxic phenomena observed by Klemperer[98] in cases of cancer, and obtained by Adamkiewicz[99] by injecting cancer-juice into animals. The latter author concludes that cancer-cells are all parasites belonging to the coccidia—an opinion with which I cannot agree on biological grounds: for distinctly intracellular parasites abound in cancers, and the host-cells have all the characters of epithelial and other tissue-cells. As stated above, I believe that the cells which invade and replace the tissues around a cancer are almost entirely parasitic, and that large collections of free parasites are met with in some cancers, thus it would not be difficult from the study of certain regions of cancers to explain Adamkiewicz's view.

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[96] Compare Nocard. *Journal of Pathol.*, June, 1893.

[97] Soudakewitch. "Centralbl. fur Bakt." Pl. 1, fig 1, No. 14-15, 1893.

Klebs[100] has recently summed up his conclusions by stating that in cancer epithelial cells become transformed into ovum-cells. To this may be raised the same general objection that applies to Cohnheim's theory of embryonic foci which become active in later life forming malignant growths. If the process of developmental differentiation were as incomplete as Cohnheim believed, or as easily reversed as Klebs suggests there would be no stability of animal form generally. The sporozoa are at certain stages singularly like ovum-cells but those present in cancer can readily be distinguished by the phenomenon of swarm-sporing as described above, by their capsules, and many other characteristics.

[98] Klemperer. 62 Versamlung Deutsch. Naturforsch. u Aerzte.
[99] Adamkiewicz. "Untersuchungen ulder den Krebs." Vienna, 1893.
[100] Klebs. 62. Versamml. Deutsch. Naturforsch.

MEDICAL DEPARTMENT

General Conclusions.

In the foregoing pages I have attempted to weave together what I have been able to verify of the work of authors, who, like myself, have recognised sporozoa in pathological formations. It is my belief that the sporozoa described by L. Pfeiffer, and the psorosperms of Wickham are but another phase of the sporozoa described by Sjöbring, Soudakewitch, &c. If I am right in this belief, the distribution of the parasites in the growth, and the constant association of the process of swarm-sporing with inflammatory changes around an epithelioma supply the most important evidence in favour of the existence of a causal relationship between parasites and growth. Although, without external evidence, it seems daring to express a definite opinion on this matter, yet when the life-history of the tissues and of the parasites is fully weighed, and the distribution of the parasites considered, I think there is sufficient evidence to justify the statement, at any rate, with regard to the commoner forms of cancer. In cancers of the breast parasites in the phases described by Sjöbring and Soudakewitch and others are abundantly present, and they afford evidence of multiplying by simple division as has been observed by L. Pfeiffer and others. In this connection the observation I have made of a similar process of the coccidium oviforme lends great support to the interpretation of these bodies as parasites. The formation of swarm-spores in cancer of the breast is more difficult to demonstrate than in squamous epithelioma on account of processes akin to that of lactation which occur in cancerous breasts. Nevertheless, I think it can be demonstrated, and, at any rate, there is abundant evidence that the pathological process is in the main the same in cancer of the breast, pancreas (Soudakewitch), liver, ovary, and other glands, as in squamous epithelioma. The same applies to cancer of the intestine (Podwyssoski, Soudakewitch) to cancer of the cervix uteri, and other cancers. With regard to certain human adenomata and sarcomata, I think I have, at least, given good reason for their being further investigated from this point of view.

The failure of many pathologists to accept the demonstration of sporozoa is not greatly to be wondered at. During the last fourteen years bacteria and bacteriological methods have become familiar, meanwhile the sporozoa, save in the hands of a few investigators, have been greatly neglected. No one who had realised the protean characters of the sporozoa would speak of the suspected cell-forms as the "cancer-body," nor would expect by special staining reactions to differentiate such parasites in the manner of, say, the tubercle bacillus.

The varied forms assumed in the different stages of their life by these organisms, until from study they become familiar, seem to have no connection with one another, and they react differently to stains. Again since the sporozoa are necessary parasites pure cultures are impracticable. At most a formation of spores is obtained. This in the well-known instance of the coccidium oviforme means a rearrangement of the body of substance of the parasites with an eightfold numerical increase of potential individuals. Such "cultures" cannot be successively repeated. Implantation experiments with this parasite have uniformly failed.

No one who is not thoroughly conversant with the sporozoa has a right to pronounce an opinion in this matter, and I feel confident no one who is conversant with them will decide the question in the negative.

It was an accident which led me to study squamous epitheliomata specially for sporozoa ; for it was in this form of cancer that I first recognised bodies of the same characters as those I had encountered in the cysts of the ureter. I was soon able to identify these bodies with those described as psorosperms by Wickham in Paget's disease and in cancer. Moreover, I was also able in squamous epithelioma to find the cell-inclusions described by Soudakewitch in glandular cancers, and to satisfy myself that these were simply other phases of the same parasites as had been previously described by Wickham, and that the bodies described by the latter author were the same as those previously described by L. Pfeiffer. When I came to examine mammary and other cancers I was soon able to appreciate the labours of Sjöbring and Soudakewitch, and in the main agree with their conclusions, and further I saw that most of the small cell-inclusions of mammary cancers and those of columnar epithelioma delineated by Podwyssoski and Sawtschenko were of the same nature as many of the bodies figured as parasites by Sjöbring and Soudakewitch. In cancer of the breast I found an explanation for the peculiar constitution of many of the cell-inclusions which appeared to multiply by simple division ; corresponding forms occurring in the rabbit's liver.[101] I looked further for the " dauerform " of the parasites, and for the mode of sporing I had observed in other cancers, and I concluded that both were present in and around the cyst-like spaces of mammary cancers. If this conclusion is correct there can be but little doubt that ordinary cancer of the breast is simply a form of psorospermosis. After much consideration I determined that, if by histological examination alone sufficient data could be obtained for a definite conclusion as to causal relationship, the squamous epithelioma was the best form of cancer to study. The cells of this form of cancer are large, and the sporozoa present of proportionate dimensions. The possibility of the sporozoa being due to a chance infection of an ulcerated surface was negatived by the fact that the bodies, I believe to be parasites, form some of the most essential parts of the growths, and include a large proportion of, if not all, Virchow's "cells of endogenous origin."

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[101] In cancer the division of the parasites is of most varied kinds, and is not identical with that shown in Figs. 23 and 25.

The discrepancies which exist between the descriptions of the parasites given by different authors are accounted for partly by the polymorphic character of the sporozoa, and partly by the incompleteness of our knowledge of this class of the protozoa, and also to errors which are inseparable from the development of a new subject. Of some structures met with in cancers from their characters alone it can be said with certainty that they are not tissue-cells but sporozoa. Others can only be known from their surroundings, others again cannot be referred definitely either to the parasitic or the tissue-cells.

If the bodies resembled no known animal forms it would be impossible to say that they were parasites at all. If they belong to known forms they are from the intracellular position some of them occupy, either suctoria or sporozoa. They have not the characters of the suctoria and they have the character of certain

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sporozoa. All authors, with the exception of M. A. Ruffer, agree that the parasites of cancer belong to the sporozoa, and there is a preponderance of opinion in favour of their being nearly allied to the coccidia. Whether or no they will prove to belong to some already known genus remains to be ascertained. The parasites of cancer are closely allied to those described above in the kidney and ureter.

I have found reasons for believing that in sporozoa living in warm-blooded animals certain stages which are well-defined in similar forms occurring in cold-blooded animals are slurred over or even altogether omitted. Indications of such a process I found in gregarinæ[102] inhabiting a tumour of a cat's lip. From this point of view it is not difficult to believe that the parasites of cancer may possibly be closely allied to Klossia, the more so when one recalls the fact that swarm-spores and lasting sporocysts may be found together in the kidneys of slugs.[103] Moreover, the difference in size observed in the parasites of different cancers has its parallel in Klossia. L. Pfeiffer[104] has found that in snails the size attained by this parasite and the number of sporogonia into which it divides depend on the size of the host-cells.

It might be objected that since some of the bodies described as swarm-spores in cancer are hardly to be distinguished from leucocytes that they cannot be proved to be of a different nature. It may be pointed out that many of these spores are readily distinguishable from leucocytes, and that minor characters such as a very high power of refraction and a coarsely granular structure distinguish others from leucocytes. How nearly certain phases of Klossia approach leucocytes in appearance may be seen by consulting Nussbaum's drawing of the sporocysts, Fig. 21, pl. viii, of Max Wolters's article.[105] By study and comparison of numerous sporing parasites it becomes evident that the bodies are not leucocytes, but swarm-spores. It is of importance to note that in some of the reticular spores each nodal point contains the elements of a living cell—nuclear matter and protoplasm, and thus it is possible that each granule of such a spore is capable of conveying infection. These granules are as small as the most minute micrococci.

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[102] Jackson Clarke, *Med. Press and Cir.*, Aug. 23rd, 1893.

[103] Ibid., *Med. Press and Cir.*, Aug. 23rd, 1893.

[104] L. Pfeiffer. "Protozoen, p. 72."

I do not think it necessary to discuss at length the strong *à priori* evidence in favour of cancer and sarcoma being modes of psorospermosis, nor to detail the positive inoculation experiments of Hanau and others. In the body, cancer has the character of an infective disease as much as has tuberculosis. It has frequently been observed to be contagious, *e.g.*, spreading from one labium majus to another, from an ulcerated cancer of the breast to the arm.

Pfeiffer and others, as well as myself, have observed among cancer-cells, movements strongly suggestive of parasites.

By comparing Figs. 32 and 33, what I believe to be the truth of the matter comes out clearly. The intracellular cells with well-marked peripheral granular layer are some of Virchow's "cells of endogenous origin," they are also identical in structure with bodies Soudakewitch has figured as parasites in cancers of the breast and pancreas. Above I have given reasons for concluding that they are sporozoa. The clusters of minute cells with distinct peripheral granule layer I believe to be some of the same parasites which have

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[105] Max Wolters, "Archives für Mikroskop. Anat." 1891.

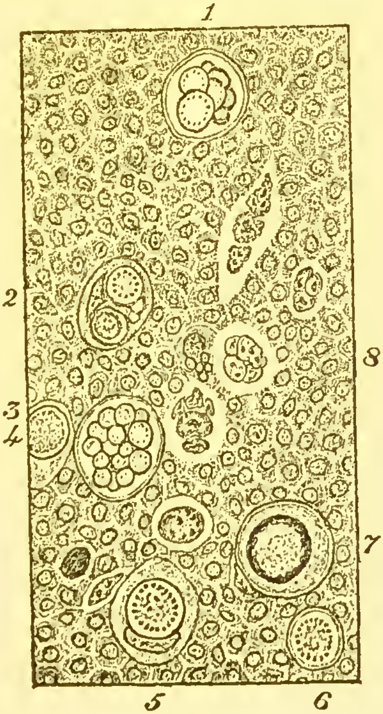

Fig. 32.—Section of a cancer of the penis. Zeiss, "F"

multiplied by direct division like the form I have described in the coccidium oviforme, see Fig. 22, p. 52, and at the same time they correspond to some of Virchow's nests of cellules. The dense body clearing centrally opposite "7" in Fig. 32 serves to link these parasites with the bodies represented in Fig. 33. These, to quote L. Wickham's[106] report on some of my Biondi-stained sections, are "more or less round bodies easily distinguished from the epithelial cells as well as from their nuclei." They constitute, I believe, L. Pfeiffer's "dauerform" of the parasite and Wickham's "psorosperms" and as shown above they may undergo division into either plasmodial or nucleated spores. It will be seen on comparing Figs. 32 and 33, that the parasites

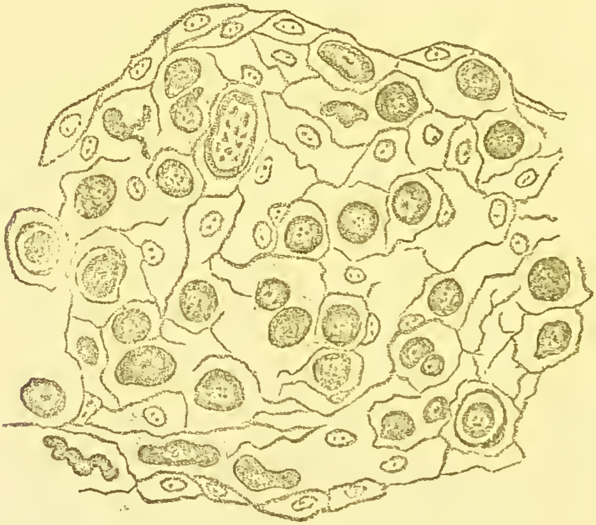

Fig. 33.—Section of cancer of the septum nasi. Zeiss, "D."

with definite granule layer occur in an actively growing part of the tumour as may be judged from the small and evidently young epithelial cells among which they lie, on the other hand the denser and sporing parasites lie for the most part in clear keratinosed epithelial cells or free. Similar differences are also to be observed in the sporozoa of breast-cancer.

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[106] L. Wickham. *Brit. Med Journ.*, May 27th, 1893, p. 1133.

Some of the irregular parasites such as are represented in Fig. 33 are quite as curious as those delineated by Korotneff.[107]

One instance will suffice to show that this view throws a new light on both the clinical and pathological aspects of cancer. Thin had already expressed a belief that Paget's disease was from the beginning a cancerous process. Darier and Wickham's researches have shown that sporozoa are present in this affection, and Wickham has found that the same parasites are present in the cancer in which the affection so often terminates. The foregoing account of squamous epithelioma and other cancers fully corroborates Wickham's view that not only are Paget's disease and cancer one and the same affection, but that both are caused by sporozoa. The importance of this from a clinical standpoint is great, since the natural history of the two diseases may now be justly assimilated. From the pathological point of view it is equally important, showing that in another class of pathological formations the distinction between inflammatory and neoplastic processes may be considered to have disappeared. Thus cancer and sarcoma are brought into still nearer relationship.

There is now so much evidence for regarding cancer as a mode of psorospermosis that even the most sceptical may take it as a working hypothesis. New lines of research and a hopeful prospect for preventive medicine are opened up.

[107] Korotneff. Centralbl. für Bakt. Mar. 23, 1893.

With regard to surgical practice only a thorough application of measures at present employed is suggested ; for cancer and sarcoma have long been treated as parasitic affections and many innocent tumours are already treated with suspicion as being likely to become malignant. There is, however, some hope held out for cases of malignant disease not amenable to surgical treatment. The various forms of ague known to be due to sporozoa, are singularly amenable to quinine and arsenic. It will be allowed that there is clear indication for practical clinical observation.

Whilst the foregoing papers have been going through the press additional papers[108] have appeared on parasitism and cancer. The paper of Ruffer and Plimmer is of great interest, and touches on many points which I hope to notice at length at some future time. There are, however, a few points which must be touched on in this place. Amongst the new figures of parasites, Ruffer and Plimmer delineate some (*e.g.*, Pl. II, Fig. 9; Pl. III, Fig. 27) with a distinct "peripheral granule layer," such as have been previously described by Soudakewitch and myself. (See *Med. Press*, Sept. 27th.) This and other points in this paper lead me to hope that the authors will soon recognise the parasites as belonging to the sporozoa.

In referring to my work Ruffer and Plimmer observe that their criticism on my specimens was practically endorsed by the "Morbid Growths Committee." This is altogether erroneous. Whilst Ruffer and Plimmer could not see in my sections bodies such as had been described by Sjöbring, Soudakewitch, Foa, and themselves, the Committee readily recognised these bodies in the same parts of the same sections I had previously demonstrated to Sims Woodhead, Ruffer, and Plimmer. But though the Committee recognised the bodies, they were unable to say whether the structures were parasites or not. In the same way the Committee readily recognised some of the bodies I had

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[108] Ruffer and Plimmer. *Journal of Pathology*, Oct., 1893. Lindsay Stevens, and Brown. *Ibid.*

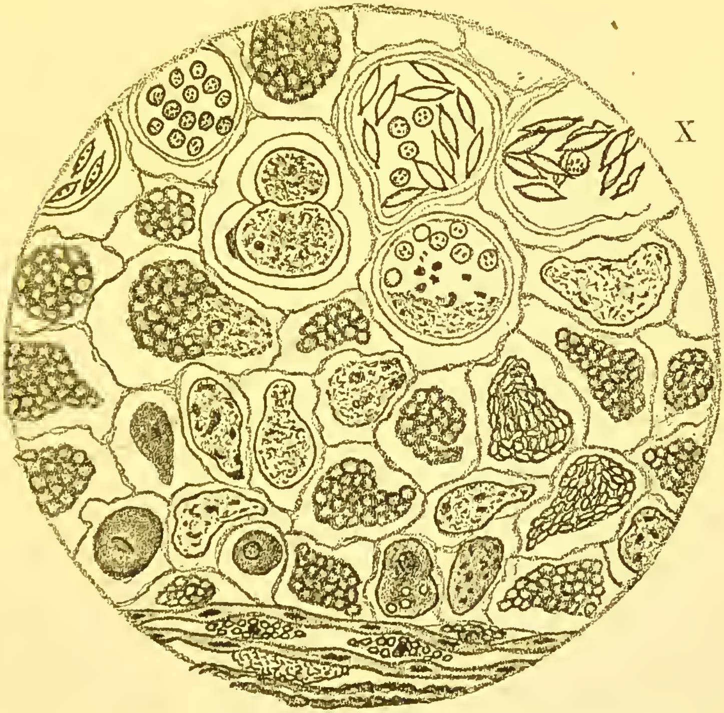

FIG. 34.—The contents of a cyst in an adenoma of a cat's lip.

described as spores, but, as with the other bodies, they were unable to say what they were.

On the strength of a brief examination of my sections of the gregarina-adenoma of the cat's lip (*Med. Press*, Aug. 23, 1893,) the authors venture to repeat their opinion and that of Prof. Boyce, that the bodies I demonstrated were not gregarinæ.

The sporogonia, pseudonavicellæ, conjugating syzygium and other bodies of Fig. 34 represent faithfully the parasites present in one of the cysts of that tumour, and the collection of bodies immediately to the left of X, comprising a typical sporogonium and several indubitable pseudonavicellæ were shown at the meeting under a very fair 1-12th o.i., and were considered by Boyce to be the eggs of a nematode.

In a note accompanying this paper the discrepancy between the illustrations of the two articles by Ruffer and Walker[109] is partly explained.

Lindsay Steven and Brown in mentioning my work state that "many competent observers refuse to recognise the structures described by Jackson Clarke as belonging to the same category as those usually regarded as parasitic organisms or inclusion-bodies, by such authors as Soudakewitch, Steinhaus, Ruffer, Galloway, &c." The foregoing papers will have made it clear that I have seen and described as parasites both the bodies described by Soudakewitch, &c., and, in addition, other bodies not described by these authors.

METHODS.

For the complete histological study of protozoa parasitic in animal tissues some power of the microscope

[109] Not completely, for one of the drawings (*Brit. Med. Jour.*, July 16th, 1892, fig. 6) is described *in the text* of that article as an infected cell modified in structure by the action of the contained parasite. I must still maintain that in the description of this figure in the two articles is evidence of a change of opinion on the part of one or both of the authors.

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higher than a $^{1}/_{12}$ o.i. is required. For this purpose I have used a Leitz's, $^{1}/_{20}$ o.i. A good condenser movable vertically is also necessary.

For fixing tissues previous to hardening, osmic acid, perchloride of mercury, or spirit, in some form or combination, suffice. In the early part of my work I placed a small thin ($\frac{1}{8}$ in.) slice of each growth in Flemming's fluid and a larger portion in a mixture of Müller's fluid and spirit (3-1), and obtained good results. I have tried Soudakewitch's method with success. Small portions of tissue are placed in 1 per cent. osmic acid solution for 24 hours, and then in running water for the same time, and next they are transferred to Müller's fluid where they are allowed to remain for from 3 to 6 days, and then hardened in 30, 60, 90, and 100 per cent. alcohol on successive days. Tissues hardened in this way are unsuitable for the Ehrlich-Biondi reagent. Foa's solution[110] consisting of equal parts of saturated solution of $HgCl_2$ in ·75 NaCl solution and 5 p. c. solution of bichromate of potassium mixed just before using, is a most convenient reagent, because the fixing is perfect and the mordant property of the bichromate gives good results with hæmatoxylin. Small portions of tissue are placed in the solution for 24 hours and then successively for the same length of time in running water, 30, 60, 90, and 100 per cent. alcohol.

Fresh teasings in glycerine are useful to correct possible phenomena due to processes of hardening. Also teasings of hardened tissue stained in bulk with borax-carmine are necessary to obtain isolated parasites and spore-cysts. For staining purposes many of the ordinary reagents should be used.

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[110] Recommended by H. G. Plimmer, *Brit. Med. Journ.* Dec. 10th, 1892.

There is no stain which differentiates sporozoa in all their stages from tissue cells. I have used chiefly well-ripened acid hæmatoxylin (Ehrlich), sometimes combined with after-staining in ·5 per cent. watery solution of eosin, and the Ehrlich-Biondi stain. For general usefulness and certainty of reaction I prefer the former, though the latter method as modified by Ruffer gives striking and demonstrative results. I obtained the Biondi-Ehrlich-Ruffer mixture from Mr. R. Kanthack to whom I am indebted for the following particulars. It was prepared from Dr. Grübler's powdered mixture of the solid dyes corresponding to Ehrlich's original mixed solution of orange G, fuchsin S, and methyl green. To 100 c.c. of a 4 per cent. solution of the powder Mr. Kanthack adds, according to Ruffer's formula, 7 c.c. of a 5 per cent. solution of acid fuchsin. If the sections are pre-stained with methyl-green a better result is frequently obtained as Ruffer has pointed out.

Some good sections can be obtained with the freezing microtome after soaking the tissue in mucilage, but where it is required to keep the contents of cysts the tissue must be embedded in celloidin or paraffin. With the former, good results are obtained with picro-carmine or hæmatoxylin and eosin, but the Biondi reagent cannot be used, and it is tedious to make serial sections; with the latter nothing gives better results than the Cambridge rocking microtome. Where the contents of cysts are to be retained sections must be fixed to the slide with glycerine and albumen before being stained. Firm growths, such as squamous epithelioma, are best freed from paraffin by xylol, and stained before being transferred to the slide.

Amœboid movement observed on the warm-stage is not of course sufficient to form a diagnosis of protozoa, since leucocytes and some tissue-cells may under suitable circumstances exhibit amœboid movement, and even undergo division when removed from the body; but when considered in conjunction with a structure peculiar to sporozoa the detection of active movement is of great importance. On the other hand, the absence of movement does not at all exclude parasites. In many sporozoa no movement has been observed, and in all it is absent at certain stages of their existence.

I have used Stricker's warm-stage heated by a current of water at 37° C., the preparations being either hanging drops or teased specimens under very thin cover-glasses.

I hope soon to extend my observations in this direction with the aid of a microscope fitted in an incubator as recommended by L. Pfeiffer.[111]

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[111] L. Pfeiffer. "Zeitschr. fur Hygiene," Bd. ii, p. 398. 1887.

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ERRATUM.

"Zeiss D" in the description of Fig. 3, should be "Zeiss A."

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